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THE FATE OF DEXTRAN IN TISSUES OF THE ACUTELY WOUNDED

A STUDY OF THE HISTOLOGIC LOCALIZATION OF DEXTRAN IN TISSUES OF KOREAN BATTLE CASUALTIES *

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Swedish workers pioneered the investigation of dextran, the polysaccharide plasma expander, in 1944 and 1945.[1] British and American studies followed. Thorsén,[2] in 1949, reported that 20,000 units of dextran had been given to 5,000 patients in Sweden. Its utilization in the United States has been restricted largely to controlled clinical and experimental studies. There now have been many reports attesting to the usefulness of dextran as a relatively short-term, blood-volume expander and to its favorable results in critical comparison with other plasma substitutes.[3-9] However, opportunities for correlative histologic studies have been rare.

Clinical Reactions. In the earlier days of dextran usage, untoward reactions, although rarely severe, were encountered frequently. These were considered referable to allergic or sensitivity mechanisms. The early Swedish dextran infusions were associated with a higher reaction rate than later products.[9,10] More highly purified dextran and a more uniform molecular size have been thought to be the important factors in reducing the incidence of reactions, so that toxic reactions are currently uncommon and of a mild degree.[7,8,11] Thorsén[12] estimated an over-all reaction rate of 0.2 per cent, with no serious reactions, calculated from 25,000 transfusion units given in Sweden. Kabat and Berg[13] demonstrated an antigenic trait of dextran by observing the development of precipitins and cutaneous sensitivity, and postulated that the occurrence of dextran in commercial sugars and its elaboration by organisms in the gastro-intestinal tract provide an explanation for systemic allergic reactions. Amspacher and Curreri,[7] in 1953, observed in reinfusion dextran studies on a large series of Army patients, some of

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whom had had initial reactions, that there were actually fewer reactions after the repeat infusions than in the initial series. None of these patients developed any demonstrable sensitivity to dextran.

More recently there has been reported a previously undescribed hemostatic defect in normal subjects after infusions of dextran.[14] This defect was characterized principally by a prolonged bleeding time. Hemorrhagic tendencies, however, were not observed in the Army-sponsored clinical trial of dextran on battle casualties in Korea.

With the gradual resolution of the side reaction problem of dextran, more attention has been directed toward its wider clinical application and the major questions have been those of its excretion, metabolism, tissue toxicity, and tissue storage.

Excretion. Dextran is known to be cleared by the kidneys fairly rapidly, but the amounts appearing in the urine of normal controls have varied considerably with the type of dextran used and the method of analysis. These values have been calculated to be in the range of 25 to 40 per cent clearance in the first 24 hours.[15] However, Lathrop and Allen,[16] by using C^{14}–labelled dextran, calculated a renal excretion of 40 to 50 per cent in the first 12 hours. Other investigators[7] have found that about 50 per cent of present-day dextran is excreted in the urine during the first 24 hours. The renal excretion then falls off so that after 72 hours it is uncommonly detected in the urine. It is also known that dextran begins to be cleared by the kidney almost immediately after the start of its infusion and the first post-infusion hours account for a marked decrease in the plasma dextran concentration. The most dramatic demonstration of dextran material in the renal tissues of experimental rats (Fig. 1) was noted in animals sacrificed 1 hour following infusion.[17] Since dextran solutions usually are composed of a rather wide range of molecular sizes, it has been reasonable to suppose that it has been the fractions of smaller molecular weight which are most rapidly excreted by the kidneys. Recently, Giebisch and Lauson[18] provided definitive data on the relationship of molecular size to renal excretion. After infusing dogs with relatively homogeneous fractions of dextran of varying average molecular weights, the average dextran/creatinine ratios of renal clearance were calculated; and the authors concluded that dextrans are excreted by a simple process of glomerular filtration without substantial tubular influence.

Granting that the majority of dextran is excreted by the kidneys, there remained the more difficult problem of accounting for approximately 20 to 40 per cent (depending largely on the average molecular weight of the infused dextran) not detected in the urine. Logically, this portion has been considered as being composed principally of the

fractions of larger molecular weight. Considerable investigation has centered about this problem and its possible relationships to metabolism and tissue storage.

Metabolism. Studies done by Lathrop and Allen[16] with C^{14}–labelled dextran indicated a recovery rate of 11 per cent of the C^{14} in the expired air (as $C^{14}O_2$) over a 4-day period. Further evidence of metabolism has been offered by an interesting experiment in which a fixed urinary dextrose/nitrogen ratio in phlorhizinized, starved dogs was elevated after the administration of radioactive dextran; and it was found that a portion of the excreted glucose, which contributed to the increased dextrose/nitrogen ratio, was radioactive.[19]

Gray and Highland[19] injected a series of mice with radioactive dextran and determined the radioactivity of total carcass fat, protein, and carbohydrate fractions. The specific radioactivity of all three tissue fractions tended to approach gradually a common value, indicating that the carbon of the glucose molecules of dextran had been incorporated into the general carbon pool of the body with a distribution in forms other than dextran. These workers concluded that the possibility of retention of dextran per se in tissues, with potential adverse effects, was no longer a valid hypothesis.

Additional proof of the ability of tissues to metabolize dextran was reported by Bloom,[20] who found that 68 per cent of dextran disappeared from the plasma of nephrectomized rats in 24 hours, as compared to 89.3 per cent for normal rats. A similar interesting observation was made in anuric battle casualties who had received dextran infusions[21] and this will be commented on later in this report.

A summarization of the experimental studies on the *in vivo* metabolism of dextran suggests that the body is capable of metabolizing the substance quite completely.

Storage. As noted previously, prior to tracing dextran in tissues with radioactive methods, there was considerable reason to suppose that the portion of infused dextran which was undetected in the urine, feces, or expired air might well have been absorbed and stored by the body. Bull *et al.*,[22] in 1949, found no chemical signs of dextran (British product) in rabbits receiving multiple injections as long as 56 days later, but did comment on serologic evidence of storage in lymph nodes and spleen. An interpretation of experiments using C^{14}–labelled dextran necessitates the consideration that the tissue assays are done by measurement of their radioactivity, which gives no assurance that the radioactive C^{14} is still bound in the intact dextran molecule. The C^{14} may well be then in a catabolite (as in $C^{14}O_2$ in expired air), or incorporated into other tissues. The more detailed experiments of Gray and

Highland,[19] previously noted, have helped to clarify such data and to de-emphasize the rôle of tissue storage as a factor of quantitative significance.

Maycock[11] has referred to minute quantities of dextran being detectable in the urine by serologic means several months after chemical analytic methods were negative. Finally, such traces of dextran were noted to disappear.

There has been experimental evidence, however, of the storage of dextran material in animal tissues. Mowry and Millican,[23] with a new technique, in 1953, reported visualization of dextran-identified material in the reticulo-endothelial cells of mice at intervals of several months following repeated infusions. Using the same method for histologic identification of dextran, I[17] have observed rather striking deposits in the spleens of rats which had received multiple dextran injections over a period of several weeks (Fig. 2).

There have been no known reports in the literature (to my knowledge) with specific reference to dextran storage in humans; however, the opportunities for such observations have been few. It is significant to mention that where dextran-identified material has been observed in the reticulo-endothelial cells of experimental animals, it has been after repeated large infusions over relatively long periods of time. More important, such foci have been unassociated with a tissue response.[17,23,24]

Histologic Manifestations of Dextran

Reported studies on the histologic manifestations and effects of dextran have been sparse. Goldenberg, Crane, and Popper[25] gave repeated infusions of a relatively unpurified grade of dextran to dogs, rabbits, and guinea-pigs. They commented on swelling and granularity of renal tubular cells with cast formation, which changes were apparently not influenced by the number of injections and which tended to reverse toward normal within 5 days following the last injection. They also described a glomerular change consisting of homogeneous, pink-staining swelling of the loops which was often severe and decreased only slightly with time following the last dextran infusion. Attempts to visualize the dextran in tissues were unsuccessful, and the authors correctly surmised that this failure to stain the substance may have been due to its being dissolved out in routine tissue preparation.

Turner and co-workers[26] in 1949, using dextran of questionable purity (10 of 30 patients had reactions of anaphylactoid type), found focal parenchymal lesions in partially exsanguinated dogs which had received dextran infusions. Reticulo-endothelial hyperplasia with the formation of giant cells was noted in the spleen. The livers of some

animals showed focal midzonal necrosis, and evidence of focal glomerular and tubular damage was seen in the kidneys. In retrospect, it seems that most of these histologic alterations were traceable to the type of dextran used, for subsequent histologic investigations have not verified a majority of these observations.

Johnston, Bennett, Lundy, and Janes,[8] in 1953, contributed interesting data on the necropsy findings in four burned patients who had received dextran (Macrodex). The only remarkable and consistent histologic change which they observed was swelling and vacuolization of the renal convoluted tubular epithelial cells. This often was associated with amorphous, eosinophilic débris within the tubular lumina. Evidence of significant pathologic findings in other organs, traceable to dextran, was absent. The renal tubular changes seemed reversible, as indicated by their being less prominent in those cases having the longest survival time following the last dextran infusion. This tended to be true, regardless of the amount administered. Johnston *et al.* referred to certain "overlapping" histologic features of the renal tubules following dextran infusions and tubular changes of lower nephron nephrosis, and they stressed the desirability of further investigation to clarify the aspects of similarity.

In this present study of necropsies on battle casualties who had received dextran, the major tubular changes referred to by both Goldenberg *et al.*[25] and Johnston *et al.*[8] were observed quite consistently. The swelling of the convoluted tubular cells, with a fine granularity and vacuolization of the cytoplasm, was identical to that of the previous descriptions and seemed to be a temporary deviation from the normal, without recognizable significance. In addition, there was opportunity to study these changes in association with a coexisting lesion of lower nephron nephrosis. No definite relationship was noted between the dextran-induced renal tubular alterations and the characteristic tubular sequences of lower nephron nephrosis. These aspects will be discussed later.

Mowry, Longley, and Millican,[24] in 1952, described a histochemical technique for the staining of dextran material in tissue preparations. In 1953, Mowry and Millican[23] observed dextran material, within 2 hours after infusions, in the blood vessels, renal tubular cells and lumina, and the liver cells of mice. The substance likewise was visible in other organs and in widely scattered phagocytes of the reticuloendothelial system after longer post-infusion intervals. Reticulo-endothelial phagocytosis was noted to persist over periods of several months following repeated infusions but in gradually diminishing amounts. Little or no dextran was seen in liver cells after 1 month. In the kid-

neys it largely disappeared from the tubular lumina in a day or so, but remained detectable in tubular epithelial cells for from 2 to 4 weeks. The authors could not determine any evidence of deleterious effects of dextran storage by reticulo-endothelial cells or any focal toxic lesions in the organs. They concluded that dextran infusions in the mouse, in almost any amount, were virtually innocuous.

Maycock,[11] in his review of plasma substitutes, cited reference to the dextran experiments of Persson[27] which showed no histologic change attributable to dextran in rabbits.

Method

In 1952, trial of dextran in battle casualties of the Korean war was approved by the Surgeon General. Satisfactory results led to limited use of dextran in Korea under the aegis of the Surgical Research Team. Liaison with the Pathology Section of the 406th Medical General Laboratory was established for histopathologic study of tissues from such patients.

The recently developed histochemical technique for localization of dextran in tissues was utilized. Mowry, Longley, and Millican[24] reported the method in 1952 and modifications were added subsequently.[23,28] The basic feature of this technique was recognition of the high aqueous solubility of dextran. By fixing tissues in absolute alcohol, processing without aqueous contact, and then staining with a modified periodic acid-Schiff (PAS) method, these workers were successful in staining dextran.

In order to gain experience with this new technique and to have histologic material as a base line for further studies on human necropsy material, a series of rats were given intravenous infusions of dextran.[17] These studies confirmed the value of the published technique and the method was adopted for analysis of necropsy tissues from battle casualties who had received dextran.

From October, 1952, until May, 1953, a special series of 15 necropsies of soldiers who had received dextran were performed in Korea. Particular efforts were made to perform the post-mortem examinations at a short interval following death. Representative tissue blocks were fixed in absolute alcohol in addition to routine fixatives. The alcohol-fixed material was processed and stained according to the modified PAS technique of Mowry and Millican.[23] Obviously this stain is not specific for dextran, for the PAS method reacts with many tissue carbohydrates and lipids. The element of confusion between these positively stained substances and dextran-related materials, however, is largely obviated by comparison with a consecutive serial control section,

stained simultaneously in the same manner, but exposed to water prior to staining. Dextran deposits, being highly soluble in aqueous solutions, are absent in the control section. The natural tissue saccharides (i.e., basement membranes, hyalin, colloid, mucin, and glycogen) are not completely soluble in either aqueous or alcoholic media and may be stained in both sections.

Some of the aspects and problems relative to the histologic identification of dextran should be mentioned. When material identified with dextran was seen in tissues, in the majority of instances it appeared as discrete purplish red granules of variable size and at times almost black. When large concentrations were present, granularity was not observed but instead solid aggregates of very dark, reddish black, homogeneous material were noted, as in the renal tubular lumina. At other times, particularly within hepatic and renal cells, the entire cytoplasm presented a rose-red stain without actual granular aggregates. Varying degrees of success were encountered in obtaining uniform staining, which, at times, defied a rational explanation, particularly when observed in identical sections of the same tissue. However, this difficulty was not of the nature of seeing "dextran-stained material" as an artefact, but rather in a tendency for dextran material to accept the stain in one area and not in another in respect to which there was no apparent reason for its not being equally demonstrable. This patchy staining affinity of dextran-containing tissues was observed likewise in the experimental rats when conditions for prompt fixation were ideal.

Observations

The following general observations were made in the histologic evaluation of dextran* in 15 necropsies of battle casualties. It should be kept in mind that this series represents a specialized group, for the average time interval from being wounded until death was about 39 hours, and the average time from the start of the last dextran infusion until death was approximately 17½ hours. This average does not include cases 5, 10, and 14 in which the infusions were given over prolonged periods. However, as noted in Table I, for 12 of the 15 patients the last infusion was in progress within 12 hours of death. These figures indicate the short temporal range of the study and suggest appropriate considerations in the interpretation of the observations.

Kidney. Dextran deposits were seen more consistently in the kidney than in any other organ studied. Likewise, dextran was seen to appear in both renal blood vessels and parenchyma with relative rapidity. In

* The average molecular weights of most of the dextran used in the clinical studies by the Surgical Research Team in Korea were 43,000 and 48,000.

TABLE I

Data upon Fifteen Casualties in Whom Dextran Was Used and the Renal Content of Dextran Graded

Case no.	Necropsy no.	Time from wound episode to death	Time from start of last dextran infusion to death	Total amount of dextran infused	Wound site	Histologic grade of dextran in kidneys	Shock status
		hrs.	*hrs.*	*cc.*			
1	J-3198	16½	6	1,000	Head	4	None
2	J-3199	32½	4½	2,000	Multiple	5	24 hours before death
3	J-3200	69½	65	1,000	Extremities	2	Entire course
4	J-3315	40½	4	1,500	Abdomen and arm	5	During last 17 hours
5	J-3426	50	47*	4,500	Burns	2	None
6	J-3430	9	6	1,500	Head	5	During last few hours
7	J-3434	6½	6	1,500	Extremities	4	Entire course
8	J-3548	6	1¼	1,500	Head and hands	1	Entire course
9	J-3694	¾	¼	500	Head	0	(Died within 1 hour of wound)
10	J-3760	17	11½*	2,000	Abdomen	3	During last few hours
11	J-3761	4½	4	500	Multiple	2	Died of hemorrhagic shock during operation
12	J-3793	106½	93½	1,500	Multiple	3	Intermittent entire course
13	J-3858	17	13	1,300	Chest	0	Terminal only
14	J-3859	42	20*	7,000	Multiple	5	During 12 hour period postoperative; none in last 20 hours
15	J-3860	167	5	1,500	Abdomen	4	Terminal 8 hours (approximately)

* Infusion given continuously until death in cases 10 and 14, and until 5 hours before death in case 5.

Histologic grading of renal dextran content:

0 = None
1 = Blood vessels only
2 = Patchy, minimal
3 = Patchy, moderate
4 = Diffuse, moderate
5 = Diffuse, marked

patients receiving large amounts within a matter of a few hours prior to death, the microscopic picture was dramatic. The entire stained section displayed a deep red mahogany color, contrasting sharply with the light pinkish red of aqueous control sections. Microscopically, the tubules contained large masses of dark positive-staining material, often completely filling the lumina (Figs. 3 and 4). These heavy aggregates were most common in the lower nephron segments and the collecting tubules. The tubular cells likewise showed staining affinity, and this was most interesting in the proximal convoluted tubules. Here, aggregates of discrete, dextran-staining granules were seen within the cytoplasm of the cells, usually unassociated with intraluminal material (Figs. 5, 6, 7, and 8). This observation likewise was made in the rat experiments (Fig. 9), corroborating the published data of Mowry and Millican[23] in mice. It is suggestive of tubular absorption or metabolism of dextran. The periglomerular spaces likewise contained granules or small clumps of the stained material.

In patients who received lesser amounts of dextran and/or at relatively long intervals prior to death, the dextran material had a tendency to be distributed in a patchy fashion and to be localized in isolated portions of the sections, principally in the convoluted tubules. This unsystematic distribution, found in other tissues as well, has seemed to be an inherent and unpredictable feature of the fixation or staining technique rather than an accurate quantitative reflection of dextran content. However, there appeared to be some degree of correlation between the amount of dextran administered and the time interval until death and the quantities seen in the sections. As noted in Table I, an attempt was made to grade the relative amounts of dextran-staining material found in the kidneys as an index for rough correlative purposes with other variables.

There was no sign of tissue damage, reaction, or inflammatory cellular response in any of the kidney sections, which could be related to the dextran. This was true also for the rat tissues, including those with multiple injections over prolonged periods.

The routine hematoxylin and eosin sections of the kidney showed the previously referred to tubular changes of cellular swelling, cytoplasmic vacuolization and granularity, and intraluminal, amorphous, eosinophilic material (Fig. 10). These findings seemed largely restricted to the convoluted portions of the nephron and, in particular, to the proximal segments. The tubular epithelial swelling occasionally was quite striking, serving to delimit sharply the convoluted tubules from the adjacent parenchyma (Fig. 11). Efforts to correlate the histologic degree of swelling of tubular cells with the quantity of

dextran administered and the involved time intervals were inconclusive on a quantitative basis. However, a rough direct relationship did exist between the amount of dextran seen in the renal tissue and the amount of tubular swelling. The fact that the tubular changes were recognized in all cases, representing a rather wide range of post-infusion intervals and dextran amounts, suggests that these alterations are produced readily and tend to disappear slowly. In none of these cases was evidence recognized of any degenerative sequences associated with this "nephrotic" picture.

Johnston and co-workers[8] commented on "overlapping" morphologic features of dextran-induced tubular changes and lower nephron nephrosis. Four of the patients in the present series had the clinical course and histopathologic picture of post-traumatic renal insufficiency (cases 2, 3, 4, and 12). The kidneys of all 4 presented the typical convoluted tubular changes which have been described, in addition to the lesion of lower nephron nephrosis. There was no particular difficulty encountered in distinguishing one lesion from the other. The major features of the so-called dextran-induced tubular changes seen in both the routine hematoxylin and eosin and Mowry PAS preparations were: (a) the sharp confinement of the lesion to the convoluted tubules (and principally the proximal portions); (b) a swollen appearance of the tubular epithelium, often with an obscuration of the precise cell borders; (c) a tendency of the cell cytoplasm to be clear and finely granular; and (d) the amorphous, eosinophilic, intraluminal material. The histologic features of lower nephron nephrosis are well known and differ from these in: (a) the location of the principal alterations (particularly distal nephron instead of proximal convoluted tubules); (b) the presence of the distinctive heme-type casts, which generally are pigmented and coarsely granular and tend to fill the lumina (in contrast to irregularly outlined islands of finely granular to wispy, strand-like masses); (c) the frequent association of tubular epithelial degenerative changes (as compared to none); and (d) the absence of impressive vacuolization and swelling of tubular cells (in comparison to these being most conspicuous in the other entity).

Interestingly enough, in the patients with associated lower nephron nephrosis, granules of dextran material occasionally were seen dispersed among the heme-cast material (Fig. 12). It is noteworthy likewise that discrete dextran-staining particles were observed within the cytoplasm of tubular lining cells, although, as in case 4, there had been a complete renal shutdown prior to a dextran infusion administered terminally (Figs. 7 and 8). These observations lead to a side issue of speculation on partial tubular function in the lower nephron syndrome.

Spleen. Dextran deposits have been described in the splenic reticuloendothelial cells of mice within short intervals following infusions.[23] In the rats used in the present investigation similar evidence was obtained, but the most definite findings were made following multiple and prolonged periods of infusion. Figure 2 is of such a spleen showing the intracellular dextran material circumferentially arranged around malpighian corpuscles. No associated tissue changes were observed. It was difficult, however, to be certain about the demonstration of minute quantities phagocytosed in the early post-infusion periods. This was likewise true for the human necropsies. On first examination, no dextran-like material was identified, but subsequent studies were suggestive of minimal focal phagocytosis; however, in no case were these deposits unequivocal. There was no recognizable evidence of tissue reaction in the human spleen.

Liver. The evaluation of dextran within the liver is complicated by the natural presence of glycogen which, being partially insoluble in both alcohol and aqueous periodic acid-Schiff preparations, appears in both slides. The dextran-staining material, however, is dissolved out in the aqueous control PAS section, and by careful comparison its intracellular and sinusoidal presence can be detected. This is often difficult, particularly when the liver cells are laden with glycogen.

In general, the human liver sections stained somewhat more intensely and diffusely with the alcoholic PAS technique than with the aqueous method. Experimentally, the hepatic concentration of dextran is more easily detected because it is possible, by fasting the animals prior to sacrificing, to remove a large portion of the confusing glycogen. Discrete phagocytosed granules of dextran-staining substance were noted also within occasional Kupffer cells of 2 cases with the longer survival periods between infusions and death. Similar localization was seen in the rat studies where it was noted most consistently in the animals receiving multiple injections over prolonged periods.

Lungs. The lungs presented a rather varied picture for dextran identification. The most common finding was that of aggregates of positive-staining granules within the blood vessels and within alveolar capillaries. There appeared to be a direct relationship between the amount of this material and the shorter time intervals between dextran infusion and death. In several of twelve cases, dextran particles occasionally were seen in the intra-alveolar spaces, sometimes intermixed with edema fluid (Figs. 13 and 14).

Pancreas. The pancreatic parenchymal cells showed no recognizable intracellular dextran-staining material. In a few cases, however, sprinklings of the positive staining granules were noted in the interstitial tissue and in peripancreatic fat.

Heart. Commonly, the myocardial capillaries were loaded with dextran granules. In only 2 cases, however, was there definite evidence of the presence of dextran granules in the stroma, and this was only patchy and light in density. The myocardial fibers, being loaded with glycogen granules, were difficult to assess without very close comparisons with the aqueous control sections. There did not, however, seem to be any localization of dextran within the muscle fibers.

Gastro-intestinal Tract. Occasional sections of stomach and intestine were submitted in the necropsy material. No dextran was identified in the walls of those organs.

Brain. Tissue blocks from the brains of 3 patients were studied. Except for the occasional sprinkling of the dextran particles within blood vessels, none was noted in the cerebral tissue.

Soft Tissues. Several of the necropsy cases and two additional surgically removed specimens included samples of striated muscle, some obtained from wound sites, and also sections of skin. Two muscle sections showed occasional foci of dextran-staining granules within the stroma. The skin sections were all interpreted as being negative save for one notable exception obtained from a severely burned patient. This will be referred to in the discussion.

Miscellaneous Organs and Tissues. Dextran-stained material was seen sporadically in the interstitial tissue of such organs as bladder, testis, and adrenal and thyroid glands (Fig. 15). As with the occasional focal deposits seen in the stroma of striated muscle, there was no predictable consistency in these findings.

Discussion

An interpretation of histologic observations in this series of 15 necropsies is limited in scope because the time intervals from dextran infusion until death were relatively short. Consequently, the possible storage of dextran in various cells or organs can be surveyed only on the basis of such changes becoming evident very early.

The tissues of these battle casualties presented some unusual considerations with reference to the normal renal clearance of dextran, for most of the patients had suffered periods of profound shock associated with extensive injuries. The Surgical Research Team at the 46th Army Surgical Hospital demonstrated, by dextran assays in the plasma and urine, the ability of these non-oliguric, severely wounded patients to begin renal clearance of dextran within an hour after the start of the infusion. Likewise, the histologic studies revealed the presence of dextran-staining material within these kidneys in the shortest available survival times.

In only 2 cases was no dextran identified in renal tissue. In one of these (case 9), the soldier received a severe gunshot wound of the head and a unit of dextran was started within minutes at the Battalion Aid Station. The patient died, however, within about 20 minutes and it is doubtful if he received much of the infusion. In the other case (no. 13), evidence of dextran in the kidneys was to be expected; failure to demonstrate it was unexplainable except possibly on the basis of a technical error in tissue preparation.

Four patients (cases 2, 3, 4, and 12) presented a particularly interesting renal study, for their kidneys showed the pathologic findings of lower nephron nephrosis. Although no previous data are available on the actual histologic demonstration of dextran in the normal human kidney, 2 of these cases (nos. 3 and 12) with renal insufficiency gave evidence suggesting a lower excretion rate. They presented the longer intervals between dextran infusions and death in the series; 65 hours and 93½ hours, respectively. Past studies on the renal clearance of dextran in normal subjects and those of the Surgical Research Team in Korea in the severely wounded without oliguria indicated that after such intervals the greater part of the substance should have been excreted. The failure of its excretion in these 2 patients may have been related to the lesion of lower nephron nephrosis. Actual granules of dextran-stained material were observed intermixed with hemoglobin casts in patients with lower nephron nephrosis (Fig. 12). An interesting corollary to these observations in cases of renal failure was the data of Howard, Frawley, Artz, and Sako[21] on dextran assays in the plasma and urine of the post-traumatic anuric soldier. These revealed a gradual disappearance of dextran from the plasma, similar to that of a non-oliguric casualty, despite renal failure. Reference was made earlier to the studies of Bloom[20] on the rat, which showed a 68 per cent disappearance of dextran from the plasma in 24 hours in nephrectomized animals. Such observations strongly support the concept of dextran metabolism, and also indicate that the kidneys probably do not play an integral rôle in the metabolic process. The visualization of dextran particles in renal tubular cells, particularly in the proximal convoluted portions, most likely indicates an absorption mechanism; and this process might be linked to the partial hydrolysis of dextran, facilitating further metabolism elsewhere in the body.

Concerning the extravascular diffusion of dextran, comment has already been made on its recognition within the interstitial spaces of some organs, where it appeared free and not within phagocytes. Two cases deserve special attention in this regard. Case 8 was a Korean private first class who received multiple penetrating wounds and was

admitted to the hospital in extremis. In addition to blood transfusions, he was given 1,500 cc. of dextran in the 1 hour and 15 minutes prior to death. Although only a minimal amount of dextran was found in the renal tissue, there were areas of marked deposition of dextran-staining granules within pulmonary alveoli (Fig. 13). The dynamics of the transference of the substance from the vascular compartment to the intra-alveolar spaces in profound shock is a matter of speculation. Similarly, whether the identified granules represent intact or altered (hydrolyzed) dextran molecules is unknown. Bollman[29] performed experiments on the extravascular diffusion of dextran from the blood in rabbits and determined that the amount of extravascular dextran was small and that, following hemorrhage, it had little influence on the mobilization of fluid available to the blood.

Another patient (case 3) was of interest in respect to the possibility of the temporary immobilization of dextran in pulmonary edema fluid. This soldier suffered multiple penetrating wounds. Despite massive blood transfusions (15,500 cc. in 6 hours), he showed a picture of shock throughout his 66-hour hospital course. He also had received 1,500 cc. of dextran in the early hours of treatment. Although there was an interval of about 64 hours from the time of dextran infusion until death, large amounts of dextran-staining granules were seen in the pulmonary air spaces. These were in association with a marked degree of pulmonary edema fluid (Fig. 14).

One of the patients (case 5) received severe burns. During his 47-hour hospital course, dextran (4,500 cc.) was administered continuously until 5 hours before death. The striking histologic finding was a heavy concentration of dextran-staining granules in the burned skin. The granules were distributed most prominently in the pools of extravasated fluid in the interstitial spaces and extended fairly deep into the corium (Fig. 16). Sections of skin of other patients failed to show dextran material. This case is of interest because of the virtual absence of dextran from all organs except the skin. The kidney revealed only minimal amounts, patchy in distribution. Renal function remained good during the patient's course; in spite of this, the dextran contained in the burned skin was not mobilized.

As previously noted, the specimens of striated muscle and skin obtained at random from the necropsy cases showed no evidence of localization of dextran in the skin, and only occasional foci within the interstitial tissue of the muscle. In addition, surgical specimens of both muscle and skin from amputation stumps were studied in 2 patients who had received dextran several hours previously. None of these tissues showed evidence of dextran localization. They were, however,

from the sites of surgical amputation and not from the actual areas of battle trauma.

The nephrosis-like tubular changes observed in kidneys following the administration of dextran were present consistently and in some cases were striking. These swollen, convoluted tubular cells presented such a distinctive picture in ordinary hematoxylin and eosin sections that their recognition was enough to warrant a strong suspicion that dextran had been administered. On consulting clinical records, this proved to be true in several cases. These tubular changes were unassociated with any cellular response or recognizable signs of local tissue reaction. As previous investigators have remarked, they appeared transient and reversible. The Mowry PAS-stained kidney sections correlated nicely with these observations; for in cases showing the most marked tubular epithelial swelling, dense concentrations of dextran-staining material were seen both intracellularly and intraluminally. Allen[30] referred to the morphologic tubular sequences incident to infusions of hypertonic sugar solutions as giving the pathologic picture of "osmotic nephrosis," a transient effect. The dextran-induced changes seem to be similar.

Concerning the reticulo-endothelial system as a storage site for dextran, the observations in this series of necropsies indicated no significant phagocytosis in either the spleen or liver. This may have been related to the short time intervals; further studies on patients with longer survival to give a greater interval between infusion and death are needed. It might be emphasized that experiments with mice[23] and rats[17] have shown definite evidence of phagocytosed dextran-staining material as long as 3 months following injection, but the relative dextran dosage in the experimental animals was high and the injections multiple. For instance, a 1 cc. dose of dextran to a 20 gm. mouse is roughly equivalent to an infusion of about 3,000 cc. for an average man. There seems little reason to expect, from the available data, that tissue storage of dextran in man will prove to be a significant factor. Even in the reported animal studies with multiple large infusions and signs of phagocytosis in the fixed reticulo-endothelial cells, no remarkable tissue changes or reactions have been recognized.[17,23]

Summary

The histologic localization of dextran in necropsy tissues of 15 battle casualties in Korea was studied.

A modified periodic acid-Schiff staining method (Mowry) was used, utilizing the aqueous solubility and relative alcohol insolubility of dextran molecules as the basic feature of the technique.

Dextran-staining material appears rapidly in the human kidney and can be demonstrated in all portions of the nephron. Intracellular granules identified as dextran have been observed in the proximal convoluted tubules of humans and of experimental rats, which suggests this mechanism as related to dextran absorption and/or metabolism.

Granules of dextran-staining substance were noted occasionally in the reticulo-endothelial cells of human liver and spleen but these were too minute and scattered for interpretive significance. However, in the rat tissues (representing multiple large infusions over long-term periods) definite and distinct focal phagocytosis was evident.

The appearance of dextran material within hepatic cells apparently occurs shortly after infusion and probably is associated with a metabolic breakdown of the dextran molecules in this organ.

There was scant evidence of the diffusibility of dextran into tissue spaces except in association with abnormal physiologic states. Specific instances of the latter were cited and included dextran immobilized in the pulmonary edema fluid of shock patients and in the skin tissues of the severely burned.

Swelling of renal convoluted tubular cells was noted to be a consistent sequel of dextran therapy. This swelling was correlated with intracellular dextran in the histochemical preparations. These changes were morphologically similar to those following hypertonic sugar infusions and appeared to be non-toxic and transient.

Four of the 15 patients had symptoms of anuria and presented the pathologic findings of lower nephron nephrosis. These were discussed from the standpoints of the effects of anuria on dextran excretion and of the significance of dextran material in the parenchyma of the anuric kidney for extended periods following infusion.

None of the tissues studied (human or experimental rat) showed any recognizable lesions referable to dextran toxicity.

The following medical officers contributed to this project by submitting necropsy tissues, clinical data, and advice: Lt. Joseph G. Strawitz, pathologist assigned to the Surgical Research Team at the 46th Army Surgical Hospital in Korea; Capt. John M. Howard, Chief of the Surgical Research Team; Lt. Col. Arthur Steer, Chief of the Pathology Section of the 406th Medical General Laboratory; Major T. R. Anderson, Assistant Chief of the Pathology Section of the 406th Medical General Laboratory; and Col. Richard P. Mason, Commanding Officer of the 406th Medical General Laboratory and the Far East Medical Research Unit.

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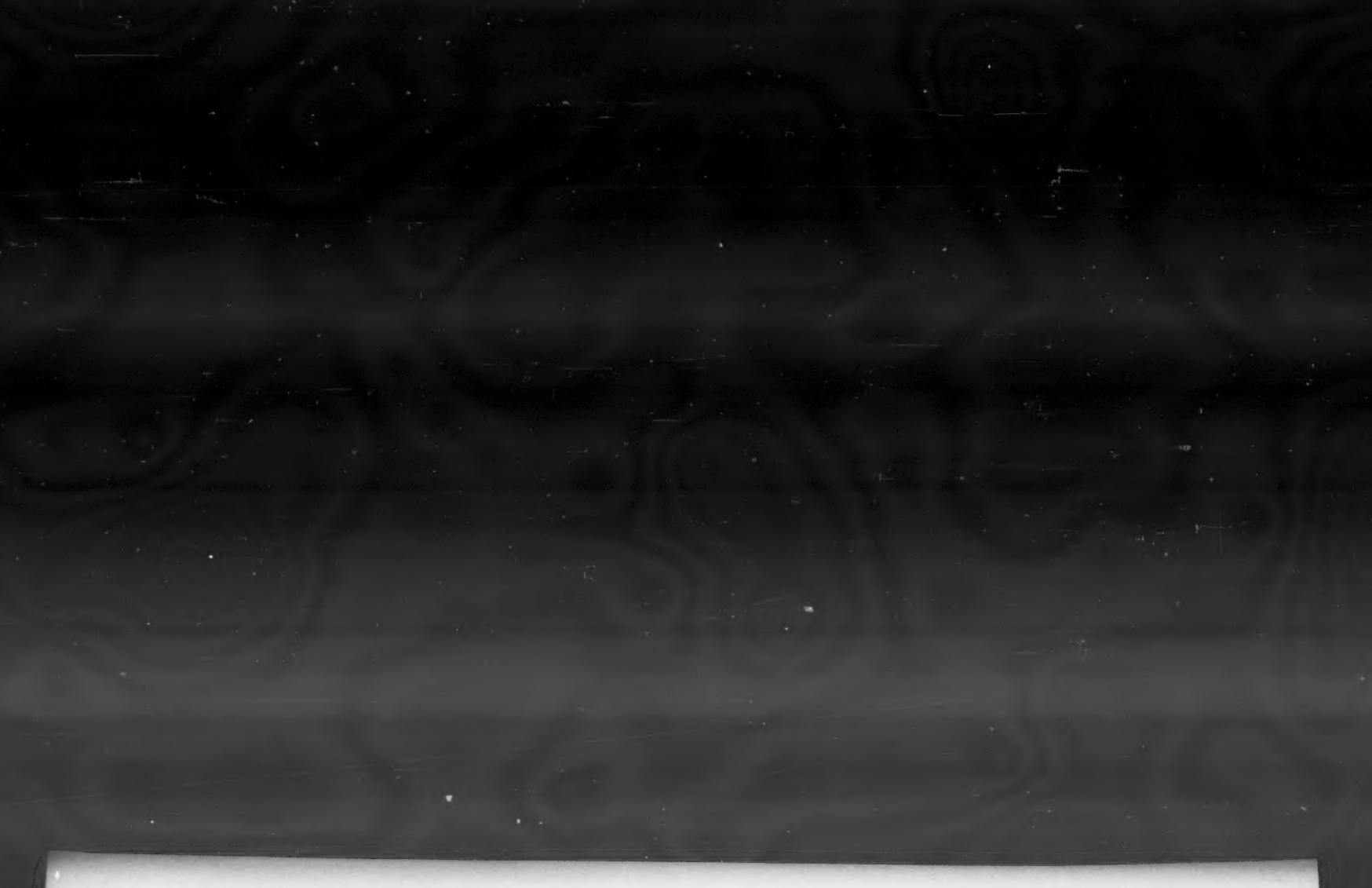
LEGENDS FOR FIGURES

FIG. 1. Rat M-6627. Low-power view of the medullary portion of a rat kidney 1 hour following a 1 cc. intravenous infusion of dextran, showing intense tubular concentration of the dark-staining dextran material. PAS (Mowry) stain. × 46.

FIG. 2. Rat M-6623. Survey view of the spleen of a rat which had received twice-weekly dextran infusions (1 cc.) over a 2-month period and then was sacrificed 1 month following the last injection. Phagocytosed particles of dextran-staining material are seen within the reticulo-endothelial cells about the periphery of the pale-staining malpighian corpuscles. These have a circumferential distribution. PAS (Mowry) stain. × 48.

FIG. 3. Case 2, necropsy J-3199. Kidney section of a soldier who died 32 hours after wounding. He received a total of 2,000 cc. of dextran during the terminal 10 hours of life, with the last 1,000 cc. starting 4½ hours prior to death. In addition to the many aggregates of dextran material in the tubular and glomerular space, the tubular cells show a marked staining affinity. PAS (Mowry) stain. × 120.

FIG. 4. Case 2, necropsy J-3199. Another renal section of the same case illustrated in Figure 3. The tubular intraluminal and intracellular localization of the dextran-staining substance is clearly shown. The large vein contains a dense concentration of dextran granules. PAS (Mowry) stain. × 120.



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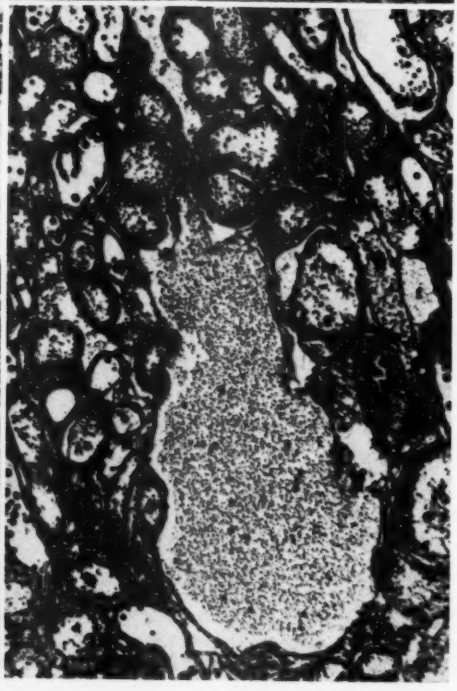

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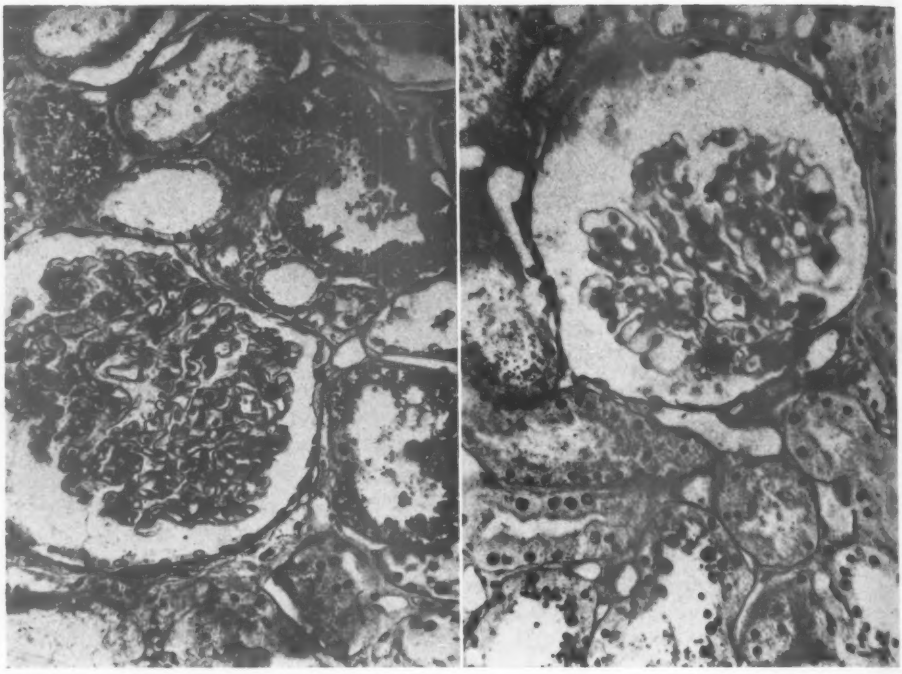


FIG. 5. Case 12, necropsy J-3793. Dextran-staining granules within the cytoplasm of convoluted tubular cells, presenting a finely stippled appearance. This battle casualty received his last dextran infusion almost 4 days prior to death. The postoperative course was complicated by oliguria (lower nephron nephrosis) and intermittent hypotension. PAS (Mowry) stain. × 240.

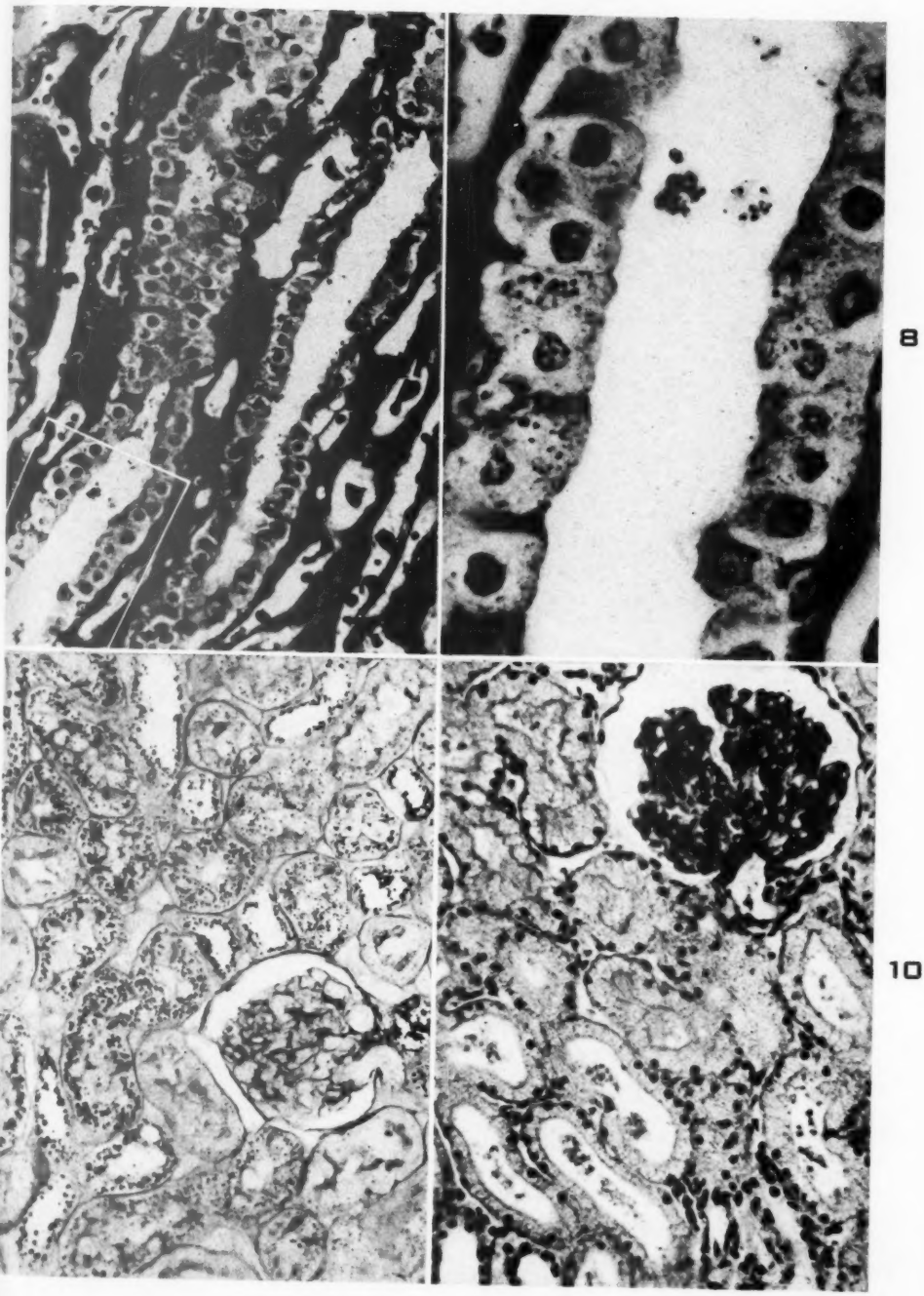
FIG. 6. Case 11, necropsy J-3761. Kidney section from a soldier who received 500 cc. of dextran at the Battalion Aid Station 30 minutes following multiple severe wounds. He died 4 hours later. Dextran-staining aggregates and granules may be noted scattered in the tubular parenchyma, both within the tubules and in the cells. PAS (Mowry) stain. × 240.

FIG. 7. Case 4, necropsy J-3315. This longitudinal view of renal tubules shows a rather diffuse sprinkling of positively staining granules within the cellular cytoplasm. The cells also appear slightly swollen. This patient received extensive wounds and developed renal insufficiency. He died 41 hours after receiving the wounds. A total of 1,500 cc. of dextran was administered: 500 cc. were given at the Battalion Aid Station and the final 1,000 cc. were infused 4 hours before death. PAS (Mowry) stain. ×240.

FIG. 8. Case 4, necropsy J-3315. This is a higher magnification of the outlined zone of Figure 7. The intracellular dextran-staining granules and the cellular swelling are illustrated. PAS (Mowry) stain. × 820.

FIG. 9. Rat 6627. Kidney section of a rat sacrificed 1 hour following a single 1 cc. intravenous infusion of dextran. (Also see Fig. 1.) The rapid appearance of the positively staining granules, not only in the distal tubular spaces, but within the proximal convoluted cells, is depicted. PAS (Mowry) stain. ×230.

FIG. 10. Case 14, necropsy J-3859. "Nephrotic" picture of renal tubular cellular swelling and vacuolization following dextran infusions. The characteristic amorphous intraluminal "casts" are evident also. This soldier suffered very severe wounds and his postoperative course, despite vigorous therapy, was marked by persistent shock. He received 7,000 cc. of dextran over the 42-hour period of survival following injury. Hematoxylin and eosin stain. × 235.



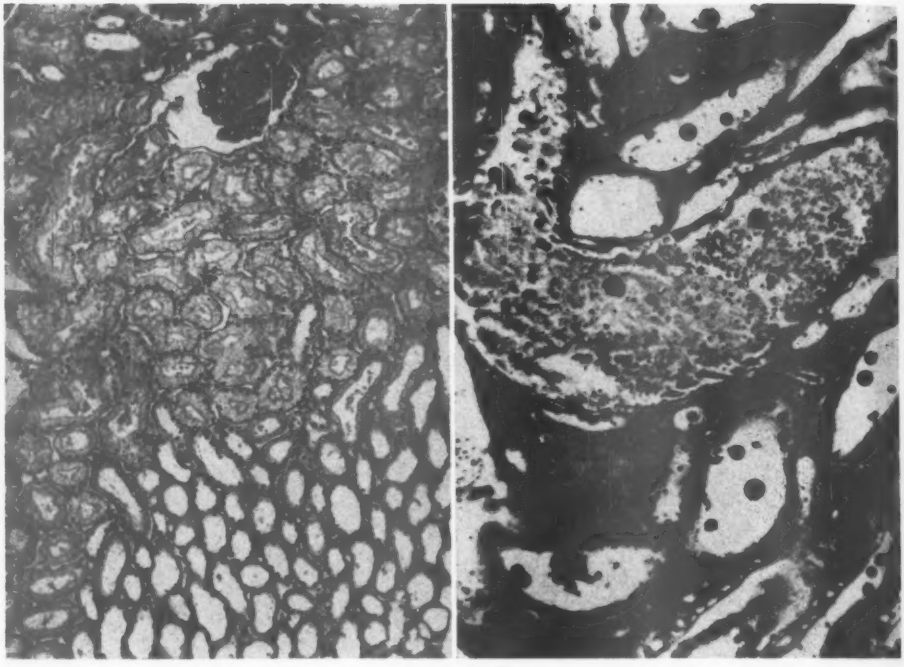


FIG. 11. Case 14, necropsy J-3859. A low-power view of the same section illustrated in Figure 10. Of note is the rather sharp confinement of the clear vacuolar swelling to the cells of the convoluted tubules. Hematoxylin and eosin stain. × 100.

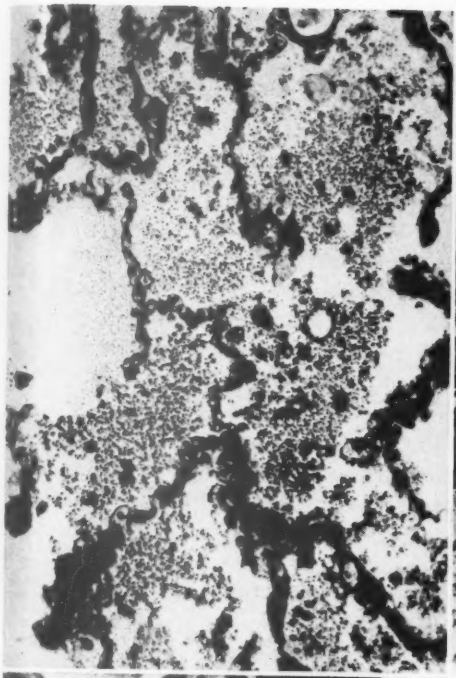
FIG. 12. Case 4, necropsy J-3315. Dextran-staining aggregates and granules in renal tubules of a patient with post-traumatic renal insufficiency. Four hours prior to death, 1,000 cc. of dextran was given. The hook-shaped tubule in the center contains a typical heme cast (the lightly staining amorphous material) in which are interspersed many dextran-identified granules of irregular size. PAS (Mowry) stain. × 240.

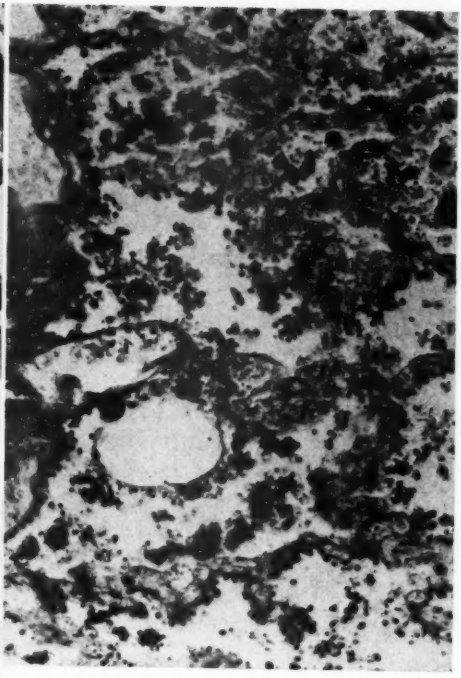
FIG. 13. Case 8, necropsy J-3548. Dextran-staining granules within pulmonary alveolar spaces. This soldier received a severe penetrating head wound, associated with profound shock. He was given, in addition to blood, 1,500 cc. of dextran during the 1¼ hours of hospitalization before death. The presence of extravascular dextran particles in the alveoli may have resulted from the shock state. PAS (Mowry) stain. × 190.

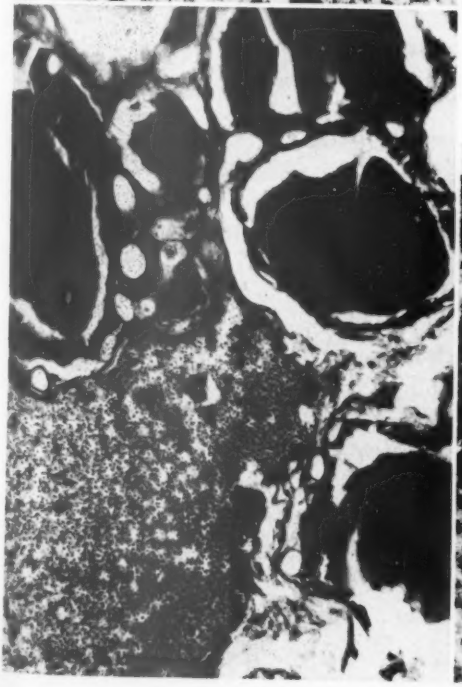
FIG. 14. Case 3, necropsy J-3200. Dextran-staining particles intermixed with edema fluid in lung tissue of a casualty who suffered from shock throughout a 70-hour post-wound survival period. This observation is of particular interest because of the long time lapse (65 hours) from the last dextran infusion. The possibility is raised of this being a manifestation of extravascular immobilization of dextran in a patient with a protracted hypotensive course. PAS (Mowry) stain. × 240.

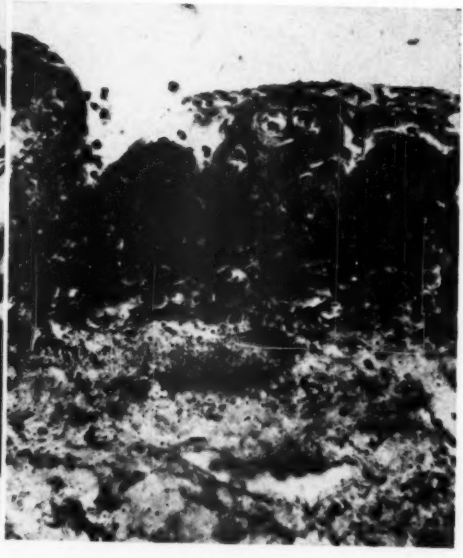
FIG. 15. Case 2, necropsy J-3199. A pool of dextran-staining granules in the interstitial connective tissue of the thyroid gland. (There was no local trauma to the neck.) This soldier suffered from marked postoperative shock, which probably was instrumental in causing this unusually prominent extravascular diffusion. A total of 2,000 cc. of dextran was administered during the last 10½ hours before death. PAS (Mowry) stain. × 240.

FIG. 16. Case 5, necropsy J-3426. This is a section of severely burned skin, which injury occurred following explosion of a stove. In addition to blood, 4,500 cc. of dextran were given over most of the 2-day survival period. Dispersion of fine, dextran-staining granules in the edematous upper corium may be noted. The other organs were either negative for any dextran-identified material or showed only small foci. PAS (Mowry) stain. × 230.









TESTICULAR SPERMATOGENIC CELL HYPERTROPHY ACCOMPANYING PROSTATIC HYPERTROPHY AND CANCER *

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There is at present no widely accepted explanation of the endocrine background of hypertrophy and cancer of the prostate. The development of hyperplasia and cancer in prostates of men who live long enough is of frequent occurrence and of great importance. It is generally stated that with age, spermatogenesis slackens and testicular interstitial cells shrink and become pigmented.[1,2] Other cytologic alterations of the testicular tubules in men with prostatic abnormalities have received less attention.

A pertinent experimental observation in rats, possibly applicable to these human diseases, consisting of the enlargement of spermatogonia, was made in the testicular tubules of parabiotic partners of animals exposed to total body x-radiation.[3] The treated rats suffered the expected severe radiation damage to gonads, with atrophy and disorganization of seminiferous tubules. During the recovery period of several days, while the testes of the irradiated animals were still quite inactive, hypertrophied spermatogonia appeared in the unirradiated testes. Spermatogenic maturation beyond this stage was slight, and cell enlargement occurred instead of the normal mitotic maturation divisions.

During this same interval a comparably striking granulosa cell hyperplasia developed in the ovaries of protected parabionts of analogous female pairs. This was explained, with endocrinologic factors known and generally accepted in females, by considering that estrogen secretion from the irradiated ovaries had decreased, releasing both pituitary glands from gonadotropic inhibition. Excessive follicle-stimulating hormone (FSH) production was inferred as the cause of the granulosa cell hyperplasia.

Controversy has been active over the existence of a second testicular hormone, termed inhibin,[4] or x-hormone[5] and variously considered to be of germinal epithelial,[4,6] Leydig,[7] or Sertoli cell[8-11] origin. Several experimental and human studies have indicated that a tubular hormone

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exists. From analogy with the explanation of ovarian changes in the parabionts, it is considered likely that the combined FSH from two rat pituitary bodies, released from testicular restraint, was responsible for the observed postirradiation spermatogonial hypertrophy.

A concurrent separate study was made of human testicular histology in patients with normal, atrophic or hypertrophied prostates and prostatic cancer. The finding that increased numbers of hypertrophied spermatogenic cells are a characteristic testicular accompaniment of benign prostatic hypertrophy suggested further inquiry into their origin. On this account the literature was searched for reports and illustrations of testicular biopsies.

Review of Literature

It was possible to find 36 reports of human testicular biopsies in which published illustrations showed increased numbers of hypertrophied spermatogenic cells, as listed in Table I. For analysis they are roughly divisible into five major groups.

1. Children and adolescents, 10 to 15 years old, with one cryptorchid testis. The descended testis characteristically had numerous hypertrophied spermatogenic elements, the cryptorchid testis none.

2. Adults, 20 to 42 years old, with pituitary adenomas of chromophobe, acidophil, or unspecified types, or hypothalamic lesions. One exception was a child 14 years old with a pituitary adenoma, treated after operation with chorionic gonadotropin, who thereafter developed hypertrophied spermatogenic cells.[15]

3. Eunuchoid or hypogonad men, particularly after treatment with gonadotropins. There were additional adults difficult to classify because of incomplete data.[7,22] In general, 17-ketosteroid excretion was low or normal. FSH excretion was variously reported as zero, low, normal, or increased.

Untreated eunuchoid men only exceptionally had numerous hypertrophied spermatogenic cells, and they were designated by such terms as fertile eunuchs[19] or as cases of partial gonadotropic failure.[22,31] Eunuchoids of the more common types and men with the Klinefelter syndrome[5,20] showed no such changes. Several men reported as having various other abnormalities of spermatogenesis had no abnormal spermatogenic hypertrophy in testicular biopsies.[32-37]

4. Older men treated with rather large amounts of estrogen, usually stilbestrol for prostatic carcinoma.[15,24-27]

5. Miscellaneous. Included were one instance each of mongolism,[16] testicular granulosa cell tumor,[28] feminizing adrenal adenoma,[29] and schizophrenia.[30] Reports of other feminizing tumors in men unfortu-

TABLE I

*Articles Illustrating Human Testicular Biopsies with Increased Hypertrophied Spermatogenic Cells, Index of Spermatogenic Hypertrophy (I.S.H.) 3.0 or More**

Author	*Author's figure*	*Age*	*I.S.H.*	*Leydig cells*	*Remarks: 17-ketosteroids given in mg./24 hrs.; FSH, in mouse units /24 hrs.*
Group 1, Hemicryptorchidism					
Sniffen[13]	4	10	5.5	Many	
Robinson & Engle[13]	5	10	5.0		
	6	11	5.5		
	7	15	6.8		
Charny *et al.*[14]	12	12	6.3	Present	
Group 2, Pituitary-hypothalamic lesions					
McCullagh[15]	4b†	14	2.0		Postoperative adenoma; CG, 36,000 u.
Conti *et al.*[16]	4	14	5.0		Fröhlich's syndrome
McCullagh *et al.*[17]	19	24	4.8		Suprasellar oligodendroglioma
	6	42	5.5		Acromegaly, eosinophil adenoma
Albert *et al.*[18]	4	28	3.5	Few	Multiple endocrine adenomas; 17-KS, 8.1; estrogen, 16‖
	2	37	7.0	Few	Chromophobe adenoma; 17-KS, 6.4; estrogen, 0
Group 3, Eunuchoids and sterility cases					
McCullagh *et al.*[19]	10‡	21	3.7	None	Eunuchoid, FSH normal, 17-KS low; ICSH low or normal
	14‡	23	6.7	Few	Same
	7	40	6.3	None	Same
Bartter *et al.*[20]	13b	21	3.0	Normal	CG, 132,000 u.; 17-KS, 9
	12§	23	5.5	Increased	CG, 75,000 u.; 17-KS, 6–9
	15§	31	5.0	Increased	CG, 300,000 u.; 17-KS, 16; positive Aschheim-Zondek test
Heller & Nelson[21]	6	26	9.0	Present	Eunuchoid; CG, 30,000 i.u.; FSH, 30 cc.
Segaloff[22]	8	26	8.0	Normal	Azoospermia; 17-KS, upper limit normal; FSH, 0
	9	45	4.0	Few	Impotent; CG, 500 i.u.
Engle[23]	2, 1 & 2	31	10.0	Normal	
	3, 2	34	4.7	Normal	
Maddock and Nelson[7]	9	35	3.5	Increased	CG, 120,000 i.u.; 17-KS and estrogen increased
	10	47	3.3	Increased	
McCullagh & Schaffenburg[6]	7	39	6.0	Few	Enuchoid, 17-KS, 4.7–4.9; FSH, 26–52
Group 4, Estrogen therapy for prostatic cancer					
de la Balze *et al.*[24]	2	58–80	6.6	Few	Stilbestrol, 10–50 mg. for 8–70 days
	5		3.0		Same
	6		4.0		Same
Schütz[25]	10	62	6.0	Few	Cyren B, 240 mg. in 4 mos.; Progynon C, 0.1 mg.
Albert *et al.*[26]	2	71	6.0	Few	Stilbestrol, large doses
Sniffen *et al.*[27]	11	?	14.0	Few	Estrogen, 45 mg. in 5 days
McCullagh[15]	7	?	10.0	Few	Stilbestrol, 200 mg. in 6 mos.
Group 5, Miscellaneous					
Conti *et al.*[16]	5	15	7.0		Mongolism
Cohen and Diamond[28]	8	21	3.6		Testicular granulosa cell tumor
Landau *et al.*[29]	5	28	7.0	Normal	Feminizing adrenal adenoma; 17-KS, 25.3; estrone, 86; estradiol, 15; estriol, 57 γ
Tourney *et al.*[30]	2	?	3.8		Schizophrenia

FSH = follicle stimulating hormone.
CG = chorionic gonadotropin. ICSH = interstitial cell stimulating hormone.
* Hypertrophied spermatogenic cells counted in 10 tubule cross-sections, total divided by 10.
† Illustrations reversed, no spermatogonial activity before therapy.
‡ Previous injections of pituitary extract.
§ Previous injections of testosterone. ‖ Units not stated.

nately did not include adequate information concerning testicular histology. Reported instances of testicular interstitial cell tumors,[38-40] or of men treated with testosterone,[33-34] had no evident increase in spermatogenic hypertrophy produced by androgenic hormones.

Data from dogs with feminizing testicular tubular tumors,[41] and from rats injected with large amounts of estrogen or gonadotropin,[25,42,43] also included references to increased numbers of hypertrophied spermatogenic components.

The Leydig cells were not described as characteristically altered in these situations. They were reported variously as absent, decreased, atrophied, normal, or relatively increased, without apparent correlation, except that the Leydig cells were uniformly few after 45 years of age and in the animals studied.

From the published animal and human material reviewed independently of the study of testicular changes accompanying prostatic disease, the following working hypothesis was developed: Overabundance of hypertrophied spermatogenic cells is a testicular tubular abnormality reflecting: (1) increased FSH levels, (2) increased estrogen levels, or (3) both. The adrenal cortex was suspected to be a richer source of estrogen than the testis, although this point is controversial since some human tumors of testicular tubular origin, for example, have been shown to produce estrogen.[44] Lipid-laden Sertoli cells remaining in tubules otherwise severely depleted might be another source of estrogen in a minority of cases of prostatic hypertrophy and cancer. Post-castration increases in FSH would favor a human testicular source of hormone inhibiting gonadotrophic secretion,[45] but the cell of origin is uncertain.

Testes in Cases of Prostatic Disease

For analysis, 50 necropsied cases with benign prostatic hypertrophy, 37 with atrophic prostates, 52 with normal prostates, 19 with benign prostatic hypertrophy and subsequent atrophy,[46,47] and 57 cases of prostatic carcinoma were collected from three hospitals in the Boston area. Sixteen necropsies of men 22 to 35 years old without prostatic disease were included as young controls. The gross weights and descriptions of the prostates, confirmed by a review of microscopic slides, were used to check the accuracy of diagnoses. Usual criteria for recognizing prostatic hypertrophy and carcinoma were employed.[48]

Testicular slides from the same cases were scrutinized, and the following features were noted: (1) spermatogenesis with production of mature sperm, recorded as abundant, moderate, slight, or none; (2) tubular tunica propria, as negative or thickened; (3) interstitial cells

of Leydig, as abundant, moderate in number, or few; (4) number and index of hypertrophied spermatogenic cells, defined as larger cells with definitely increased amounts of eosinophilic or clear cytoplasm, compared to the normal elements.

As to the spermatogonia, the diameters of hypertrophied cells measured from 25 to 35 μ, compared to 15 to 20 μ for normal cells. Nuclear diameters were about two thirds of the cell diameters, and chromosomes were either in prophase or dispersed as in resting cells (Figs. 1 to 4). Sertoli cells were distinguished by their elongated cell outlines, the oval nuclear shapes, a relative paucity of nuclear chromatin, and prominent nucleoli. Enlarged spermatogonia were found in tubules at levels normally occupied by primary and secondary spermatocytes, ordinarily two cell diameters distant from the basement membrane. Their nuclei and cytoplasm corresponded to the description and illustrations of large spermatogonia given by Roosen-Runge and Barlow,[49] or to gigantic spermatogonia reported by de la Balze *et al.*[24]

While it was at first thought that all the hypertrophied intratubular cells were spermatogonia, application of the cytologic observations of Knudsen[50] on perfectly fixed bull testes showed that reasonable numbers of abnormally swollen cells were the hydropic primary spermatocytes he described. Perhaps one third to one half of the hypertrophied cells, with diameters of over 30 μ, located three or more cell diameters from the tubular walls, were considered to be spermatocytes, arrested in prophase and with hydropic cytoplasmic swelling. Polyploidic spermatocytes, described in bulls by Knudsen[51] as having reconstituted nuclei about 12 μ in diameter and a cell size of 60 to 70 μ, were less common in human cases, and contributed 5 to 10 per cent of the hypertrophied cells. In human testes the polyploidic cells measured 45 to 60 μ in diameter (Figs. 5 to 8). Multinucleated cells, ascribable to incomplete mitotic division,[32] were observed in only one case.

Hypertrophied spermatogonia and spermatocytes generally had the nuclear structure of resting or prophasic cells, and formed the most superficial layer of spermatogenic elements. They were not free floating, or sloughed into tubules. Estimation of their number was made by counting the hypertrophied spermatogenic cells in 10 tubular cross sections. This number, divided by 10, was the index of their frequency, which varied from 0 to over 10. When both testes were available, the indices were averaged; in fact, they differed by an average of 0.3 for 39 pertinent cases.

Most testes, except those with tubules completely hyalinized or lined only by vacuolated Sertoli cells, had some hypertrophied cells. In otherwise normal testes from necropsies, the number ranged to a

maximum level of 2.5 per tubular cross section. Over 3 hypertrophied spermatogenic cells per tubular cross section was considered to be an abnormal increase, although more extensive counts on multiple sections might well show 1.5 per tubular cross section to be the upper limit of normal. Young men dying suddenly, or with normal testicular biopsies, often had very few hypertrophied cells, but the average index for the 16 necropsy cases 35 years of age or less was 2.3.

Results

Benign Prostatic Hypertrophy. Spermatogenesis was absent or slight in 41 of 50 cases (82 per cent) of benign prostatic hypertrophy. The surrounding tubular tunica propria was abnormally thickened in about half the testes. Interstitial cells were few in 32 of 50 testes (64 per cent). Likewise, hypertrophied spermatogenic cells were increased in 38 of 50 (76 per cent). Tabulation of the results is given in Table II.

Table II

Histologic Features of the Testes of Patients with and Without Prostatic Disease

Condition of prostate and number of cases	Index of spermatogenic hypertrophy*					Interstitial cells		
	0	0.1–2.9	3.0–3.9	4–5	Over 6	Many	Moderate	Few
Benign prostatic hypertrophy, 50 cases	2	10	21	8	9	4	14	32
Atrophy, 37 cases	10	13	10	3	1	1	11	25
Benign prostatic hypertrophy with subsequent atrophy, 19 cases	0	6	0	7	6	1	6	12
Carcinoma, 57 cases	11	10	11	10	15	1	14	42
Negative, 52 cases	10	30	7	3	2	7	21	23

* Method of determination as given in Table I and text.

Prostatic Atrophy. In 32 of 36 testes from cases with prostatic atrophy, spermatogenesis was very much reduced or absent (89 per cent) and thickened tubular tunicas accompanied about the same proportion. In 25 of 37 testes (68 per cent), interstitial cells were few. Fourteen of 37 had abnormally increased hypertrophied cells (38 per cent), and this lower incidence represented the outstanding difference observed in association with atrophy in contrast to hypertrophy of the prostate. In 19 additional cases with atrophy supervening after hypertrophy,[46,47] little or no spermatogenesis occurred in 16, and 17 had a thickened tubular tunica propria. Twelve had few interstitial cells, and 13 had increased numbers of hypertrophied cells (69 per cent).

Prostatic Carcinoma. Spermatogenesis was markedly reduced in 39 of 57 cases of prostatic carcinoma (69 per cent). In 73 per cent of 55 cases thickening of the tunica propria occurred. Few interstitial cells were seen in 42 of 57 cases (74 per cent). In 36 of 57 cases (63 per cent) there were increased indices for hypertrophied spermatogenic cells.

Normal Prostate. Thirty-three of 52 cases with normal prostates had reduced spermatogenesis (64 per cent). Thickening of the tunica propria occurred in slightly over half of the group. In 23 of 51 cases (45 per cent), interstitial cells were few, and 12 of 52 cases (23 per cent) had abnormally numerous hypertrophied cells.

Various diseases are well known forerunners of diminished spermatogenesis. Senile alterations are believed to be responsible for some thickening of the tunica propria.[52] Tabulation of all cases by age also suggested a gradual decrease in the numbers of Leydig interstitial cells with increasing years.

Examination of the prostates containing carcinoma showed an accompanying benign hypertrophy in 37 of 43 cases (86 per cent). The other 14 prostates had their architecture obliterated by neoplastic overgrowth. It was concluded that most prostate glands containing carcinoma showed morphologic indications of an antecedent hypertrophy, not necessarily involving the lobes or regions where the carcinoma subsequently developed.[53]

Discussion

Spermatogenic hypertrophy represents a category within the broad term of spermatogenic maturation arrest, also sometimes termed tubular disorganization or incomplete spermatogenesis.[16,22,23,45] Apparently this tubular abnormality has not been described previously or discussed as a separate entity. But for the indications from the experiments with parabiotic rats,[3] it might not have been recognized in the present study.

Why spermatogonia and spermatocytes failed to undergo mitosis or reduction divisions and instead acquired increased amounts of clear cytoplasm is unknown. The cytologic changes included glycogen storage[24] similar to that characterizing the response of cervical epithelium to estrogen. In this respect hypertrophied spermatogenic elements resembled an endocrine target organ rather than a source of hormones. The neoplastic cell most closely simulated was that of the seminoma, which is usually believed not to produce any significant hormonal secretion, although occasionally accompanied by a positive pregnancy test,[54] perhaps because of intermingled choriocarcinoma.[55]

For reasons from the literature as already detailed, animal and human testes with increased numbers of hypertrophied spermatogonia usually had evidences of stimulation by excess gonadotropin, chiefly FSH.

Investigations of testicular aging have indicated a gradual decrease in number of interstitial cells and presumably in androgenic function.[2] If decreased testosterone secretion were not accompanied by any other endocrine changes, it appears likely that the prostate would remain unaltered or would atrophy. More commonly, when the interstitial cell functions had waned, significant estrogenic effects were evident. This was inferred from the presence of numerous hypertrophied spermatogenic cells, such as were found in 76 per cent of men with prostatic hypertrophy. Benign prostatic enlargement was not attributable simply to unopposed estrogen effects, since therapeutic doses in castrate males have led to prostatic atrophy and squamous metaplasia.[56,57] More likely, the combination of decreased androgens, relatively increased estrogens, and possibly other adrenal cortical steroid hormones was accountable for the development of nodular prostatic hypertrophy. Teilum,[8] and Moore[47] and others have reached a similar conclusion by different approaches.

It would appear that, as in women who developed breast tumors[58] and as in some experimental animals,[59,60] changes of gonadal endocrine secretions had allowed an abnormal expansion of pituitary and secondary adrenal cortical hormonal functions to stimulate the growth of target organs, particularly accessory sex glands.

Prostatic carcinomas, arising in glands with antecedent hypertrophy, at times had the testicular stigmas of previous estrogen effects. In about two thirds of the cases with cancer, estrogenic stimulation apparently was continuous up to the time of death or orchiectomy. In the remaining one third, any estrogenic effects had waned, and this cancer group resembled, in its testicular histologic features, cases with prostatic atrophy following benign enlargement. Atrophic regions in the posterior prostatic capsule are believed to be particularly susceptible to the development of cancer.[48,53]

Further investigations are in prospect to examine other endocrine glands, particularly the adrenal and pituitary, for indications of these or other abnormal activities. The belief of Mellgren[61] that adrenal enlargement is a characteristic accompaniment of prostatic hypertrophy deserves more study. From information collected in the present project, suspicion has been directed to the pituitary and adrenal cortex as the most important endocrine glands in the background of both benign and malignant prostatic growths.

Summary

Hypertrophied spermatogenic cells have been illustrated in published studies of the testes of men with inferred excessive pituitary gonadotropic (follicle-stimulating hormone) or estrogenic stimulation, and in experimental animals in comparable endocrine states. Testes from men with abnormal or normal prostate glands showed relatively increased numbers of hypertrophied spermatogenic cells in 76 per cent of the cases with prostatic hypertrophy, 63 per cent with prostatic carcinoma, and in 23 per cent with normal prostates. Estrogen secretion, suspected to be partly of adrenal cortical origin following pituitary stimulation, is discussed as a significant factor in the development of prostatic hypertrophy and cancer.

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LEGENDS FOR FIGURES

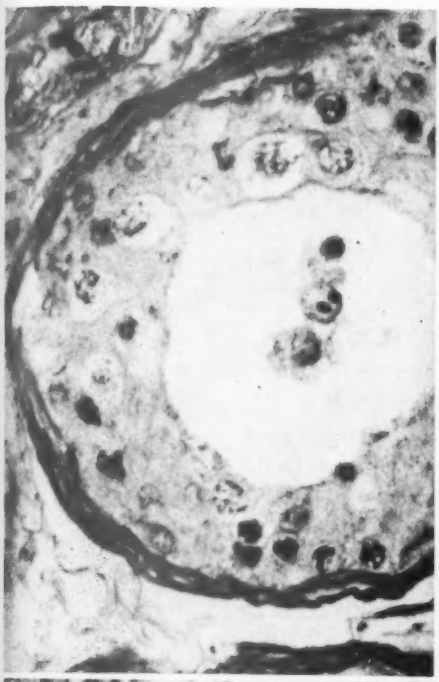
All sections were stained with hematoxylin and eosin. × 500.

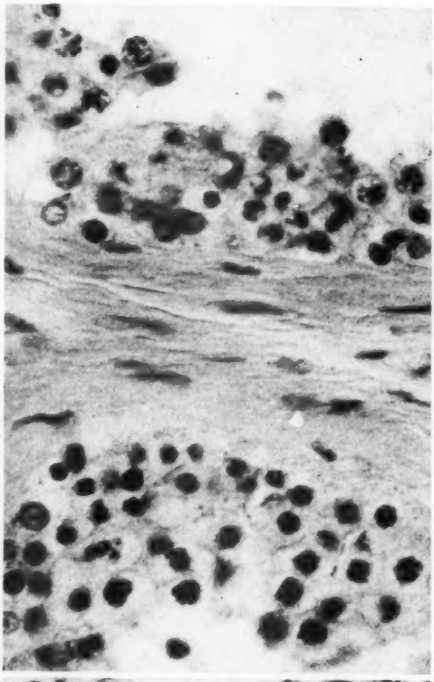
FIG. 1. Hypertrophied spermatogonia with swollen hydropic cytoplasm, from the testis of a 49-year-old man with early benign prostatic hypertrophy (45 gm.).

FIG. 2. Hypertrophied spermatogenic cells, including spermatocytes. Tunica propria is thickened. From an 82-year-old man, whose prostate weighed 80 gm. and showed nodular hypertrophy. An epidermoid carcinoma of the larynx was present.

FIG. 3. Hydropic and hypertrophied spermatogenic cells, from a man 87 years old, surgically castrated.

FIG. 4. Testis from a man 50 years old, without abnormal numbers of hypertrophied tubular cells, for comparison with Figures 1 to 3. The prostate weighed 30 gm. and appeared normal.





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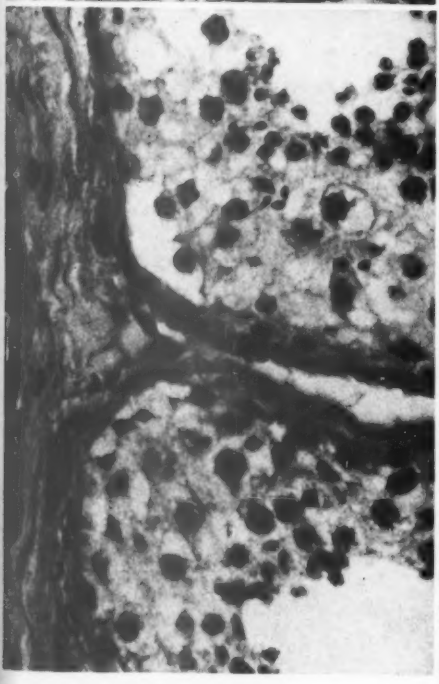

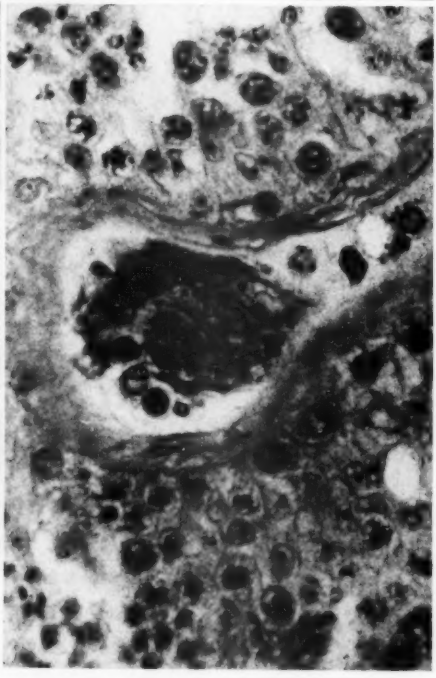

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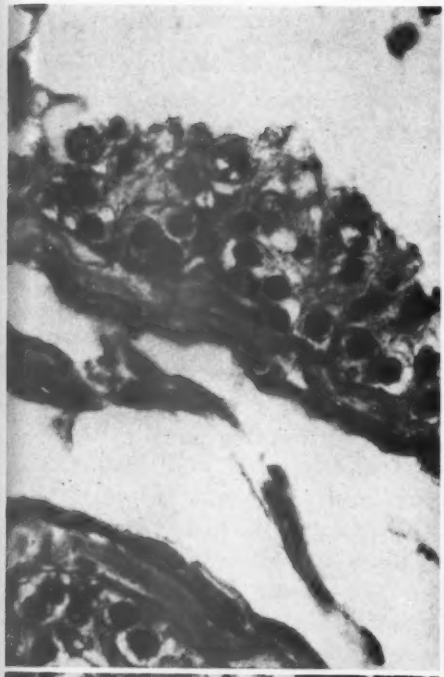
FIG. 5. Hypertrophic spermatogonia and polyploidic spermatocytes from the testis of a 75-year-old man with prostatic adenocarcinoma.

FIG. 6. Similar enlarged and abnormal spermatocytes from a man 65 years old with prostatic hypertrophy and adenocarcinoma.

FIG. 7. Hydropic polyploidic spermatocytes, from a man 58 years old with adenocarcinoma of prostate.

FIG. 8. Abnormal spermatogenesis with numerous multinucleated spermatocytes and a few hypertrophied cells, from a 67-year-old man with adenocarcinoma of the prostate. The uninvolved portion of the prostate showed no abnormality.



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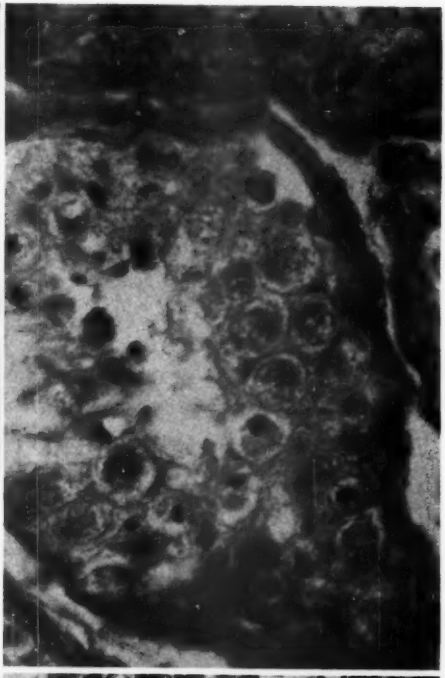

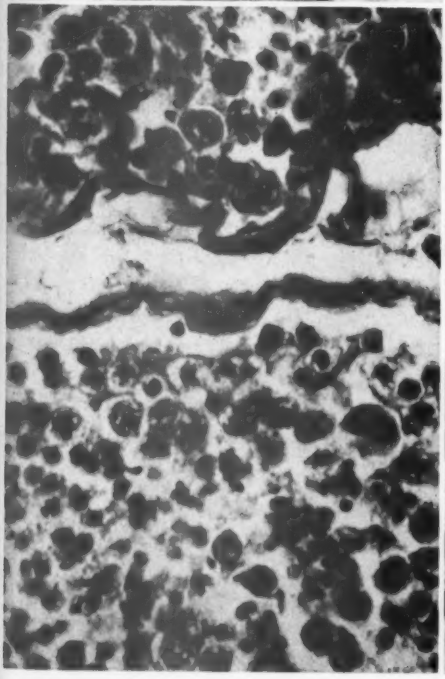

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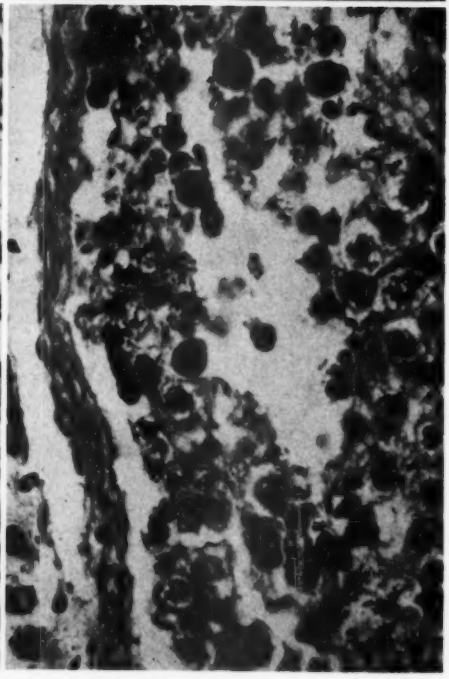

MACROFOLLICULAR LYMPHOMA *

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The unusually long course attributed to macrofollicular lymphoma has led some authors to doubt its malignant character, and hence to confusion in nomenclature. Study of the present series of 136 cases has shown the prognosis to be less favorable than that sometimes assigned. This tumor, with few exceptions, must be regarded as malignant from its onset. To investigate the apparent discrepancy, the problem has been approached from two viewpoints: firstly, that of differentiation from hyperplastic lymphoid conditions, and, secondly, of evaluation of the extent to which macrofollicular lymphoma can be considered a disease entity in view of its frequently reported alteration in structure, or so-called malignant transformation.

The sundry designations—lymphoma, lymphosarcoma, lymphoblastoma, lymphadenopathy, reticulosis, and hyperplasia—indicate the continued existence of considerable disagreement. These terms usually are preceded by an indication of the large size of the follicles: giant follicle, macrofollicular, or, merely, "follicular." The follicles certainly are usually large, but not always so, and perhaps the prefix "giant" is not strictly appropriate. Macrofollicular lymphoma is a term which is widely used and there are no serious objections to its continued use, as "lymphoma" usually has a malignant connotation.

Macrofollicular lymphoma often is specified as affecting both lymph nodes and spleen, and Baggenstoss and Heck[1] stated that the spleen was usually greatly enlarged. Jackson and Parker,[2] however, considered splenomegaly to be less frequent, a finding in accord with this study.

Material Data

In a general review of histologic material of lymphomas and hyperplastic lymphoid tissue, 136 cases of macrofollicular lymphoma were encountered, as well as 14 other cases, which will be considered separately in which the diagnosis rested between hyperplasia and macrofollicular lymphoma. The original diagnosis in these latter cases had been either definite or probable macrofollicular lymphoma. It has been possible to follow 130 of the first group (21 with necropsy) and all of the second group, extending as far back as 25 years. The cases

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are from the files of the New England Deaconess Hospital, the New England Baptist and Pondville Hospitals, the Lahey Clinic, and the Harvard Cancer Commission.

So-called benign lymphoma of the rectum, some follicular lymphoid conditions of salivary glands, and benign lymphocytoma cutis have been excluded since there is much doubt whether they are neoplastic, or merely reactive hyperplasia.

Macrofollicular lymphoma is a disease of middle age, and no patient in this series was under 25 years (Table I), the average age being 52.8 years. Examples in children require particularly careful scrutiny before they can be accepted as authentic. Jackson and Parker[2] had no patient under the age of 20 in their series of 39 cases. Gall *et al.*[3] found an average of 50 years in their 63 cases, as against 41.7 years for 507 cases of lymphoma generally, exclusive of the follicular type. Sex incidence in my series was 72 males and 64 females.

Duration of symptoms before biopsy was ascertained in 112 cases, and the great majority (Table II) had a very short history indeed: 1 year or less in 95 cases. The median duration was 4 months and the average 9 months. Extreme variations may occur; the longest duration of a tumor before biopsy was 17 years.

TABLE I

Age at Time of Biopsy in 136 Cases of Macrofollicular Lymphoma

Age in years	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89
No. of cases	0	0	6	15	35	34	33	11	2

TABLE II

Duration of Symptoms Before Biopsy

Years	0–3/12	4/12–1	1 1/12–2	Over 2
No. of cases (112)	53	42	12	5

At the time of the first biopsy, in 38 cases the disease was apparently localized to a single peripheral nodal region, such as one cervical, axillary, or inguinal site.

In 79 cases, two or more separate sites were involved and, for practical purposes, this will be called a generalized process. Six patients with retroperitoneal tumors also will be included in this group, making 85 cases in all. In the other 13 cases the degree of involvement was not known.

The more common sites were neck and groin, with 10 cases each.

Of 23 generalized cases in which the initial site of glandular enlargement was known, the more common were again neck and groin, with 12 and 11 cases, respectively.

Initially, of the 136 cases, 116 had peripheral lymph node enlargement; 12 had abdominal tumor, namely, rectum (1 case), small intestine (2 cases), stomach (1 case), and retroperitoneal tissues (8 cases); and the others had tumor located in "thigh" (2 cases), scalp and parotid gland (1 case), tonsil (2 cases), and roof of mouth (1 case). In 2 the site was not known.

Histologic Features

Macrofollicular lymphoma in its typical form (Fig. 2) has a highly characteristic appearance, and in the earlier stages often closely resembles hyperplastic lymphoid tissue. The tumor also appears in infinite variations of the type lesion. Characteristically, the follicles are large, rounded, and packed together, with little intervening tissue, and they are numerically increased. They have a discrete central portion, usually making up most of the follicle and resembling the germ center of the non-neoplastic node. This has a surrounding rim of packed small lymphocytes. The interfollicular tissue is compressed and lightly infiltrated by lymphoid cells.

When the tumor first arises it would appear to have some degree of multifocal origin within varying numbers of pre-existing lymph follicles, whether this be restricted to a single lymph node or not. In large part, however, the tumor is formed by the production of new follicles which, theoretically, could evolve from multipotential tumor cells by either a multifocal process, direct spread of tumor, or by metastatic dissemination. In an inflammatory lesion, non-neoplastic lymph follicles with germ centers may appear almost anywhere in the body in sites where lymph follicles are not normally present. In a comparable way, the follicles of macrofollicular lymphoma may arise as multiple, apparently separate entities (Fig. 1). Careful examination, however, suggests that in a large proportion of cases the disease is a diffuse process; the interstitial tissue separating the follicles is also part of the neoplastic process. While this may not be readily apparent in some areas, elsewhere it may be quite obvious, the tumor presenting as a diffuse lymphosarcoma which is producing follicles formed of cells differing from those of the basic tumor (Fig. 3). Gall *et al.*[3] described this appearance and featured it in their Figure 1. The interstitial cells usually are somewhat less mature than the typical small lymphocyte. Even when one lymph node shows a multifocal appearance with the follicles separated by apparently non-neoplastic tissue, other nodes

removed at an earlier or later date, or even at the same time, may show diffuse lymphosarcoma with follicle formation, with follicular and intervening tumor perhaps equally prominent. In view of this, the disease must be considered essentially a diffuse process; that is, a lymphoma capable of differentiating into follicles.

As would be expected, the follicles may be present in any proportion to the intervening tissue and they are nearly always larger than normal. They may be large and closely packed, or small and numerous (Fig. 4) and may be irregular in distribution. Usually rounded, the follicles may be altered in shape by mutual pressure; in some instances, however, they may assume peculiar configurations (Fig. 5). The follicular outline often is quite discrete, but it may be poorly defined, or may sometimes give the appearance of having ruptured, with spraying out of the follicular cells into the interstitial tissue. Confluence of follicles may develop and is a common late phase (Fig. 6). In the early stages the cellular structure of the follicles shows the same slight pleomorphism and lymphocytic infiltration as in non-neoplastic follicles and there even may be phagocytosis of nuclear débris by occasional cells. Phagocytosis, however, was an infrequent phenomenon and never as prominent as in hyperplastic lymphoid tissue. Pleomorphism usually persists through the more typical examples, and the more malignant-looking follicles usually are made up of pleomorphic sarcomatous tissue. At this stage, occasional giant cells commonly are seen among the follicular cells and they may be of Hodgkin's disease type, either multinucleated or with lobulated nuclei (Fig. 7). The surrounding rim of packed small lymphocytes usually ceases to be evident and we have follicles, or rather "germ centers," of obviously malignant tumor set in a lymphomatous background. The resemblance to the usual non-neoplastic lymph follicles now is not so apparent, but the analogy with normal functional lymphoid tissue is still evident. Conway,[4] in discussing the cyclic changes in lymphatic nodules produced by infection, described a stage in which the germ centers are "bare" or devoid of a peripheral zone of small lymphocytes. Occasionally, the cells of the follicles may assume a somewhat epithelioid character. Rotter[5] defined six types of non-neoplastic germ centers, one of which was the epithelioid type.

As we have a whole range of gradations, all of which may be seen in a large series of cases and even in individual cases, it is reasonable to link them as a single disease process—macrofollicular lymphoma. Discussion of necropsy material will make this even more evident. As will be described later, some of the tumors were associated with diffuse lymphosarcoma completely devoid of follicles. It should be stressed

that in this series no tumors have been included unless they were at some time in their course predominantly of follicular type and in them the follicles were quite obvious.

Nature of Macrofollicular Lymphoma and Its Differentiation from Hyperplasia

"Macrofollicular lymphoma" must be considered to be a tumor which, with few possible exceptions, is malignant. The follicles might well be interpreted merely as multiple foci of tumor set in non-neoplastic lymphoid tissue, but as they may so closely mimic non-neoplastic lymph follicles it would seem that they are a functional manifestation of a malignant lymphoma. In their more differentiated forms the follicles are regular in pattern and distribution, and show the concentric arrangement of the more peripheral cells commonly seen in non-neoplastic follicles, and also noted by Baggenstoss and Heck.[1] The neoplastic pseudo-germ centers arise both in pre-existing non-neoplastic follicles and *de novo*. Several lymph nodes which were only partly disorganized by macrofollicular lymphoma clearly demonstrated the origin in existing follicles. In other portions occasional follicles showed changes in the germ centers identical with those in the disorganized part and dissimilar to those in the remaining normal follicles (Figs. 8 and 9).

The most difficult problem with macrofollicular lymphoma is its differentiation from markedly hyperplastic lymphoid tissue. Both macrofollicular lymphoma and hyperplastic lymphoid tissue may show large, irregularly shaped and distorted follicles, sometimes of quite bizarre appearance (Fig. 10). This can not, therefore, be regarded as a very reliable point of differentiation, since, if anything, it is more apparent in hyperplastic glands. Numerical increase of follicles may be more obvious in the lymphoma than in the hyperplastic gland. Mitotic figures in the follicular cells were noted in both hyperplastic and tumor process. The presence of macrophages containing particulate matter, presumably nuclear débris, and scattered in varying numbers among the follicular cells is indicative of hyperplasia (Fig. 11). It is rare in macrofollicular lymphoma and in this series was particularly prominent in only 2 cases, which presented a real problem since both patients had generalized lymphadenopathy when first seen and both later died from lymphoma, one after 1 month and the other after 2¼ years. In biopsy material from lymph nodes some of the follicles appeared to be hyperplastic, with numerous macrophages in the germ centers, and others neoplastic, without macrophages. A further difficulty is the fact that phagocytosis is not invariably a prominent feature

of non-neoplastic follicles, and so it is of value as a differential point only when present.

We are left, therefore, with a small proportion of cases which could not be correctly classified. Jackson and Parker[2] stated that uniformity of the follicular cells, lack of phagocytosis, and invasion of the capsule of the node all favor the diagnosis of macrofollicular lymphoma. Murray and Broders[6] studied sections of lymph nodes from 516 cases of various kinds and found capsular invasion by mature lymphocytes largely absent from their "non-inflammatory and non-neoplastic" group, but present with about equal frequency in their inflammatory and malignant lymphoma groups.

In addition to the macrofollicular lymphoma series are the 14 cases (Table III) in which lymph node biopsy was reported at the time as

TABLE III

Fourteen Cases Considered to be Lymphoid (Giant Follicular) Hyperplasia

Case	Sex	Age	Site	Duration before biopsy	Follow-up
				yrs.	*yrs.*
1	M	13	Axilla	2/12	17
2	F	67	Neck	Not known	12 2/12
3	F	46	Axilla	Not known	11 8/12
4	M	22	Neck	2/12	10
5	M	42	Axilla	1 6/12	9
6	M	46	Axilla	Not known	8 11/12
7	F	28	Neck	6/12	8
8	F	28	Groin	5/12	4 3/12
9	F	60	Neck	5/12	4 3/12, died
10	F	23	Groin	1/12	3 5/12
11	F	54	Neck	4/12	3
12	F	17	Neck	4/12	2 1/12
13	F	72	Submaxillary region	9/12	1 3/12, died
14	F	75	Axilla	2/12	11/12, died

either definite or probable macrofollicular lymphoma and in which the appearance is probably that of hyperplasia, but macrofollicular lymphoma cannot be excluded completely. This group might well be labelled giant follicular hyperplasia in that the follicles are large. However, the group would not appear to have clear-cut limits and the use of this term would serve only to maintain the present confusion in terminology.

The follicles were very irregular in 3 instances and less so in 6 others. Macrophages containing particulate matter were numerous, prominent, and visible even under low-power magnification in the follicles of 11 cases. In the other 3 they were present but search had

to be made for them. The follicles usually were not as closely packed as in macrofollicular lymphoma, but some variants of the latter may present this appearance, at least in some parts of the tissue. It is noteworthy that in all these cases the lesion apparently was localized and that no patient subsequently developed recurrence, though followed for periods as long as 17 years (Table III). Three did in fact die, but from causes other than lymphoma.

Baggenstoss and Heck,[1] in an investigation of 13 cases of macrofollicular lymphoma, also examined 50 hyperplastic lymph nodes for comparison. Their differential criteria could not be satisfactorily applied in the present investigation. Evans and Doan[7] reported 16 cases of "giant follicle hyperplasia," in 4 of which the patients subsequently developed sarcoma. The authors used the term synonymously with giant follicle lymphoma, but most of their cases appear to be analogous to my hyperplastic series and their illustrations clearly show numerous prominent phagocytic macrophages in the germ centers. There is no specific mention of the structure of the germ centers in the 4 examples which became malignant, but the authors emphasized that the clinical course and individual prognosis may be predicted fairly accurately by identification of the several morphologically and functionally differing follicular cell types.

It would seem from the literature and from experience with the present series of cases that hyperplasia often is diagnosed as macrofollicular lymphoma and that cases of early macrofollicular lymphoma are sometimes believed to be hyperplasia. The former is apparent, for example, in some of the cases reported by Wetherley-Mein *et al.*[8] and by Evans and Doan.[7]

The association of rheumatoid arthritis with hyperplastic lymph glands, which may simulate macrofollicular lymphoma, is now well established.[9] Motulsky *et al.*[9] stated that lymph node involvement is believed to be part of rheumatoid arthritis as a systemic disease rather than a regional process secondary to drainage from diseased joints, but they did not deny the possibility of local reaction, particularly with enlarged nodes at the more unusual sites. Saxen,[10] in his case 9, described an example of polyarthritis with enlargement of cervical and axillary nodes in which biopsy showed giant follicle hyperplasia. The nodes regressed and the patient was well 4 years later. Two patients (cases 5 and 6) of my hyperplastic group (Table III), one with rheumatoid arthritis and the other with a monarticular arthritis, had lymphadenopathy which was apparently localized and related to affected joints. In each instance macrofollicular lymphoma had been diagnosed histologically and yet both patients were alive 9 years later.

According to Gall and Stout,[11] the lymph nodes in infectious mono-

nucleosis may show pronounced hyperplasia of the follicles, with numerous mitotic figures and evidence of phagocytosis; in the later phase the enlarged follicles may become irregular in shape.

Most authors consider "macrofollicular lymphoma" to be a malignant lymphoma, but some express doubts as to its initial nature. Even recent authors, such as Leonardelli and Bertogalli,[12] still consider the histologic picture of "giant follicular lymphadenopathy" to be not an entity, but a change caused by a variety of factors, including inflammatory and toxic stimuli. In the first description of macrofollicular lymphoma, in 1925, Brill, Baehr, and Rosenthal[13] applied the term giant lymph follicle hyperplasia, but, by 1927, Baehr and Rosenthal[14] had decided that it was malignant. Symmers,[15] in 1938, still retained his original non-neoplastic view, put forward in 1927, although conceding frequent transition into tumor. Advocates of the malignant concept include Baehr and Klemperer,[16] Jackson,[17] and Gall *et al.*[3]

The possibility that there may be a benign form of macrofollicular lymphoma cannot be excluded entirely. Some of our patients with localized tumor apparently were cured, although histologically the picture (Fig. 12) could not be distinguished from that seen in some of the fatal cases. Again, some of the hyperplastic series might also be reconsidered. Willis[18] divided macrofollicular lymphoma into localized benign lymphoma and a multiple malignant form with or without splenomegaly. He described the localized variety as affecting a single lymph node, usually in the neck. Certainly, in some of our "cured" cases that had had localized disease, the tumor was solitary, that is, it affected a single lymph node or a tonsil; but, in others, several nodes were so affected. The question of the existence of benign forms of lymphoma is highly controversial, owing to this difficulty in distinguishing them histologically from malignant lymphoma on the one hand, and hyperplasia on the other.

There are other facets of this same problem in such conditions as so-called benign lymphoma of the rectum, some follicular lymphoid conditions of salivary glands, and benign lymphocytoma cutis.

In the light of conclusions derived from this study, a contemporary series of 17 cases of benign lymphoma (lymphoid polyp) of the rectum has been reviewed. Most of the lesions were macrofollicular and in nearly all there were prominent phagocytic macrophages in the follicles. The lymphoid tissue mass tended to be of fairly discrete outline (Fig. 13) and somewhat lobulated. In this series all stages could be seen from obviously non-neoplastic lymphoid tissue to one particular example which was virtually indistinguishable microscopically from macrofollicular lymphoma (Fig. 13). In this instance there was no

obvious phagocytosis. With one exception, it was the only example which did not show this feature. Follow-up of these 2 cases and of 3 of the most neoplastic appearing of the others failed to show any recurrence after 16, 8, 7, 6, and 6 years respectively. In 6 of 10 cases for which history was available, the lesion co-existed with hemorrhoids. In the past and even currently the label macrofollicular lymphoma or lymphosarcoma often has been applied to these rectal lesions and yet follow-up has failed to reveal any case which developed a malignant course. Most lesions of this kind would seem to be examples of lymphoid hyperplasia. In the single example of macrofollicular lymphoma of the rectum of the original series the patient had a hard mass half-encircling the bowel 4 inches from the anus. Biopsy showed tumor which infiltrated among the mucosal glands. The patient later developed evidence of generalized lymphoma and a lymph node removed from the neck showed similar follicular structure.

Some lymphoid conditions of salivary glands may simulate macrofollicular lymphoma and yet run a benign course. In addition, macrofollicular lymphoma in the usual malignant sense may involve salivary glands as part of the tumor process, which occurred in 2 cases of the series, or even primarily.

Benign lymphocytoma cutis, sometimes referred to as localized Spiegler-Fendt sarcoid, shows varying degrees of the follicular pattern, and may be benign lymphoma, but is probably usually reactive hyperplasia of lymphoid tissue.

Developmental Sequence of Macrofollicular Lymphoma and the Relationship to Other Malignant Lymphomas

Interrelationship of the lymphomas and transitions between one type and another have come into some prominence and acceptance in recent years. There is no doubt that a close relationship between types does exist, although the degree of overlap is a matter of some dispute. Macrofollicular lymphoma would appear to be the very hub of this problem since it is alleged to change into any of the other lymphomas —lymphosarcoma, reticulum cell sarcoma, leukemia, and Hodgkin's disease. It certainly is an unstable tumor which almost invariably loses its follicle-producing powers, becoming a diffuse malignant lymphoma. Study of a large series of cases, with individual biopsies, sequential biopsies, and biopsy with subsequent necropsy, reveals all stages of the possible developmental sequence.

At once a difficult problem is met—the identification and labelling of the cells forming the various types of malignant lymphoma. The malignant lymphocytoma or lymphocytic lymphosarcoma, formed of

small lymphocyte-like tumor cells, and the reticulum cell sarcoma as interpreted by Oberling[19] (1928) can be identified reasonably well. There is a large inbetween group which will be labelled lymphoblastic lymphosarcoma, but it should be emphasized that the exact identity of these cells is a matter of conjecture.

This very considerable confusion is reflected in the distribution figures of malignant lymphoma, exclusive of Hodgkin's disease, formulated by several investigators. Sugarbaker and Craver,[20] from a total of 196 cases, labelled 184 (94 per cent) reticulum cell sarcoma, 3 malignant lymphocytoma, and 9 giant follicle lymphoma. Warren and Picena,[21] using Oberling's[19] more restricted criteria for reticulum cell sarcoma, found only 11 examples in 308 cases (3.6 per cent). Stout[22,23] divided his 164 cases into 89 with reticulum cell sarcoma, 55 with lymphocytic lymphosarcoma, and 20 with giant follicle lymphoma. He considered lymphosarcomas with cells having a diameter less than twice that of a normal lymphocyte as lymphocytic lymphosarcoma, and those with larger cells as reticulum cell lymphosarcomas. Jackson[24] stated that there will be a proportion of 100 cases of reticulum cell sarcoma to 60 of lymphosarcoma and perhaps 50 of giant follicle lymphoma. It will therefore be seen that controversy hinges around the identity of cells more immature than the small lymphocyte—the lymphoblasts and reticulum cells. Probably in any case, the picture may be mixed.

Reference to Table IV will show the various forms of diffuse malignant lymphoma encountered in 21 cases of macrofollicular lymphoma which were necropsied. They include a further type of diffuse tumor which was present in 8 cases. This was a polymorphous cell sarcoma in which multinucleate cells were common and, in the more anaplastic variants, contained large, bizarre, giant cells (Figs. 14 and 15). This tumor corresponds in many ways to Hodgkin's sarcoma and pleomorphic reticulum cell sarcoma, and apparently usually arises from follicular cells.

Macrofollicular lymphoma seldom maintains its follicular form throughout the course of the disease. Cohen and Bergstrom[25] described such a case, in which the follicular pattern persisted even though the tumor was widespread at necropsy. No similar example was encountered in this series but several were follicular in part at necropsy.

It will be seen from Table V that only 8 of the 30 cases with multiple biopsies showed a consistent follicular pattern. The others all showed diffuse lymphoma at some time in their course. At necropsy (Table IV), this was even more evident in that the tumor in all 21 cases apparently was predominantly non-follicular.

The usual developmental sequence is for macrofollicular lymphoma to lose its follicular pattern and change into a diffuse lymphoma. The mechanism by which this is brought about is usually by coalescence of the follicles with varying degrees of both expansion of the interstitial tumor and loss of distinction between it and the follicular tumor. Another possibility is for follicular tumor to merge with interstitial tumor, that is, spraying out of the follicular cells into the surrounding neoplastic tissue. Or the follicles may simply disappear, with overgrowth of the interstitial tumor.

There are thus three phases or pictures which we might bear in

TABLE IV

Varieties of Histologic Structure Present at Necropsy in 21 Cases in which Biopsy Had Shown Macrofollicular Lymphoma

Case	Macrofollicular	Lymphocytic lymphosarcoma	Lymphoblastic lymphosarcoma	Polymorphous cell sarcoma
1	+		+	
2	+		+	
3	+	+	+	
4	+	+		
5	+	+		
6	+			+
7				+
8				+
9				+
10			+	+
11			+	+
12		+	+	+
13		+	+	+
14		+	+	
15		+	+	
16		+	+	
17			+	
18			+	
19			+	
20			+	
21		+		

mind. First, there is the macrofollicular lymphoma. In the second, sheets of lymphoblastic or polymorphous cell sarcoma apparently are produced by follicular fusion and set in a background of lymphocytic lymphosarcoma. These sheets are of varying size and shape, and may be fairly discrete or in some places may merge with the interstitial tumor. There may even be some residual follicular pattern, but on the whole a diagnosis of macrofollicular lymphoma cannot be made, only

surmised. In the third phase, there is a diffuse sarcoma without any distinguishing features.

Review of the 21 post-mortem cases revealed that only 6 showed a persisting follicular pattern and in each this was in fact associated with various forms of diffuse malignant lymphoma, as shown in Table IV. The picture at necropsy may present only one or any combination of the three phases just described. With respect to the second phase, a

TABLE V

Change in Structure of Macrofollicular Lymphoma Observed by Multiple Biopsies and Necropsy

Case	Biopsy							Necropsy
	1st	2nd	3rd	4th	5th	6th	7th	
1	1929, F.*	1943, D.†						
2	1930, F.	1932, F.						
3	1934, F.D.‡	1944, F.D.						
4	1934, F.	1934, D.						
5	1936, F.	1940, F.						
6	1937, D.	1937, F.	1940, D.	1940, D.	1941, D.	1938, D.		1948, D.
7	1937, D.	1939, F.						
8	1938, D.	1939, F.						
9	1939, F.	1939, F.	1939, F.					
10	1941, F.D.	1949, D.						1949, D.
11	1941, F.	1945, F.						
12	1943, F.	1944, D.						
13	1943, F.	1953, D.						
14	1944, D.	1947, F.	1948, F.					
15	1944, D.	1945, F.						
16	1944, D.	1949, F.	1952, F.D.	1952, F.D.	1952, D.	1952, D.	1952, D.	
17	1945, D.	1947, F.D.						
18	1946, F.	1947, D.	1947, D.					1947, D.
19	1946, F.	1948, D.						
20	1947, F.	1948, F.						
21	1947, F.D.	1949, F.	1950, F.	1953, F.	1955, F.			
22	1948, F.	1948, D.						
23	1949, F.D.	1953, D.	1953, D.					
24	1950, F.	1953, D.						
25	1950, F.	1950, D.						
26	1951, F.D.	1952, F.	1954, D.					
27	1951, F.	1952, F.						1952, D.
28	1952, F.	1952, F.						
29	1952, F.	1952, F.						
30	1953, F.D.	1954, F.D.						

* F.=follicular. † D.=diffuse. ‡ F.D.=follicular and diffuse.

picture occasioned by the dual character of macrofollicular lymphoma, sometimes it is possible to recognize at necropsy two distinct types of growth, often in different sites. One is diffuse lymphocytic or lymphoblastic lymphosarcoma of fairly regular pattern. The other is diffuse polymorphous cell sarcoma of follicular origin, similar in many ways to the more sarcomatous forms of Hodgkin's disease, and going on to an anaplastic giant cell sarcoma. Cells of Hodgkin's type, either multinucleate or with lobulated nuclei, are common in this tumor, with bizarre giant cells in the more anaplastic versions. Exactly the same picture may be seen also in the follicles of macrofollicular lymphoma; in fact, all stages leading up to sarcoma of this polymorphous type were observed within the follicles and, in some, the expansion and fusion of follicles could be seen clearly to form a region of such a tumor.

In none of the cases in this series was macrofollicular lymphoma seen to change into the "granulomatous" form of Hodgkin's disease. In one biopsy specimen of otherwise quite typical macrofollicular lymphoma a small focus of characteristic Hodgkin's disease was found, situated in the interstitial tissue between the follicles and distinct from them. Hodgkin's disease, although easy enough to recognize histologically in its more typical appearances, becomes more difficult to delineate as its classical features become fewer. Histologic definition thus is difficult owing to the ill defined limits, within which many of our tumors could be fitted. Custer and Bernhard,[26] in a large series of 1,300 cases of lymphoma including Hodgkin's disease, observed that 2 cases of macrofollicular lymphoma underwent transition to Hodgkin's sarcoma, one to Hodgkin's granuloma, and one to Hodgkin's paragranuloma. Symmers[27] stated that transformation of macrofollicular lymphoma to Hodgkin's disease does occur. Held and Chasnoff[28] were of like opinion, but an editorial comment indicated lack of belief that any well authenticated case of macrofollicular lymphoma could have terminated in Hodgkin's disease.

At least 5 of our patients developed chronic (small cell) lymphatic leukemia, with high white blood cell counts ranging from 40,000 to 250,000 per cmm. In 4, this was discovered when the patient was first seen, and in the other terminally, 4 years after initial diagnosis of macrofollicular lymphoma. For only 93 cases were figures available for at least one blood examination; thus the incidence would be at least 5 per cent. More extensive hematologic investigations might well have increased the incidence. Symmers[15] reported 4 cases. Gall *et al.*[3] had not encountered a single example in their 63 cases, but subsequently observed such a case.

Contrary to earlier views, the spleen is not always enlarged at necropsy. Heintzelmann,[29] in a review of the literature in 1946, stated that all patients who were necropsied had shown splenomegaly, sometimes even in high degree. Of 20 of our necropsy cases, the spleen was enlarged in only 13. In 4 it weighed more than 1,000 gm., the largest weighing 3,220 gm. Histologically, tumor was found in the spleen in 10 of the 13 cases, and was of diffuse type, except in 2 instances in which it was macrofollicular.

Macrofollicular Lymphoma as a Disease Entity

Macrofollicular lymphoma generally is considered to be a form of lymphoma which is easily recognized providing it can be distinguished from hyperplasia, and a condition which tends to be maintained for a long period, to terminate eventually as a malignant lymphoma of diffuse non-follicular type. While the follicular pattern is maintained, it has been considered that the prognosis is good.

However, the possible histologic ramifications of this disease must be realized. When the tumor changes to a diffuse form it can no longer have any specific course attributable to its previous follicular pattern. Furthermore, we must bear in mind that the disease usually is generalized when the patient is first seen, and initial biopsy of a single lymph node will not reveal necessarily the character of all. This could even apply to patients with localized disease if there are multiple nodes involved. It of course applies to any type of lymphoma, but is especially true of the macrofollicular variety since it is such an unstable tumor, as is clearly revealed by its almost inevitable eventual alteration in structure with loss of the follicular character. Naturally, therefore, we can suppose that if more than one node were examined, some might show a non-follicular diffuse lymphoma. To investigate this point cases were selected in which more than a single lymph node or organ was removed from a single site at the same time and examined histologically, or in which nodes from different sites were excised at the same time.

Distinct and separate regions of macrofollicular lymphoma and of diffuse lymphosarcoma were, in fact, found in the same lymph node in 13 cases. There were 11 cases in which macrofollicular lymphoma was found in one node or organ and entirely diffuse lymphosarcoma in another, both having been removed on the same occasion. In 2 such instances the nodes were from widely different sites. One patient had three nodes excised from the right groin, the "back," and the "axilla." These showed, respectively, typical macrofollicular lymphoma, diffuse lymphosarcoma, and diffuse lymphosarcoma. The second patient had

one node, which showed macrofollicular lymphoma, removed from the groin, and another, showing diffuse lymphosarcoma, removed from the axilla.

Although the examples are necessarily random samplings, macrofollicular lymphoma seen in a single node in generalized lymphadenopathy cannot necessarily be assumed to portray the character of the whole tumor process.

That macrofollicular lymphoma is a disease entity is not in doubt. It would indeed be remarkable if this prominent feature of the normal lymph node, the lymphoid follicle, was not reproduced in some tumors of lymphoid tissue. From the lability of the normal non-neoplastic follicle it should be expected that macrofollicular lymphoma would prove to be an unstable tumor, more so than other members of the lymphoma group, which have no great tendency to change their character apart from a tendency to become more anaplastic during their course.

In those cases in which both macrofollicular lymphoma and diffuse lymphoma have been found, whether in the same lymph node, in different nodes removed at the same occasion, or in sequential biopsies, we have to question whether the diffuse lymphoma has arisen from macrofollicular lymphoma or whether the macrofollicular lymphoma is an associate or side development of a diffuse lymphosarcoma. It is true that in some instances there is evidence that the diffuse lymphoma is of macrofollicular developmental origin. We may, therefore, see sheets of lymphoblastic lymphosarcoma or polymorphous cell sarcoma in a background of lymphocytic lymphosarcoma, or even see an occasional tumor follicle. There is little doubt, however, that this is not the complete answer, and it is in some of these cases that one might doubt that the diffuse lymphoma is of macrofollicular origin. In support of this concept we find that 7 of the 30 cases listed in Table V had an original biopsy diagnosis of diffuse lymphoma. As a further possibility, macrofollicular lymphoma and diffuse lymphoma may arise simultaneously. Case 3 (Table V) appears to be an example; both biopsies, taken in 1934 and 1944, showed exactly the same picture of part macrofollicular lymphoma and part diffuse lymphoma, without any indication whatever that the diffuse tumor may have arisen from follicular tumor.

Macrofollicular lymphoma, therefore, is usually an entity, with characteristic natural progression into diffuse lymphoma. This development may occur at any time and so it is impossible to attach individual prognostic connotation to the disease and only generalizations can be made. It would seem also that in some cases, macrofol-

TABLE VI

Survival from Time of Biopsy in 130 Cases of Macrofollicular Lymphoma

Number of cases	Survival in years	Under 1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–15	15–20
	Patients dead (87)	25	19	11	7	7	5	2	3	2	1	5	0
	Patients still living (43)	15	6	6	6	2	2	0	2	1	1	1	1

licular lymphoma may be part of a diffuse lymphoma arising *de novo*. This would be in keeping with the small degree of overlap which apparently exists between the individual tumors of the lymphoma group.

PROGNOSIS

A general idea of survival figures for the whole series of cases may be gathered from Table VI. The 5-year survival rate was calculated both from the beginning of symptoms and from the date of biopsy, but the figures are almost identical as would be expected, since the median duration of symptoms before biopsy was only 4 months. Taken from the date of biopsy, there were 26 5-year survivals of a total 72 cases (36.1 per cent), including 6 cases (8.3 per cent) probably cured, and 20 with persistent disease (27.8 per cent). Corresponding percentages for 3-year survivals (98 cases) are 49, 12.3, and 36.7. The median survival of all patients, both living (43 cases) and dead (87 cases), was 2 years (Table VI).

The extent of disease when the patient is first seen is of vital importance and the biggest factor in evaluating prognosis. No patient with generalized disease, in this series, has been cured, although a number have survived with the disease for many years; in fact 5 survived for more than 10 years, including one who died from lymphoma 14 years after diagnosis by biopsy.

In all, 114 of our patients had generalized disease, either when first seen, or developing at a later date. Of these, 83 came within the 3-year follow-up category (7 were still alive, but with disease) and the average survival was 3 3/12 years from the time of first biopsy.

The over-all results are less optimistic than those reported so far, which are based on relatively small series. Wetherley-Mein *et al.*,[8] in a review of the literature, found a total of only

208 cases of follicular lymphoma up to 1952. Gall and Mallory[30] found 53 per cent of 5-year survivals and an average total duration of 5.6 years in 38 cases of macrofollicular lymphoma. Baggenstoss and Heck[1] found that the mean duration of life after onset of the disease in their 6 patients who died of the disease was 6.3 years. In a review of the literature, and including their own cases, they found the average duration of 29 patients, who had died, was 4.8 years. More recently, Shimkin *et al.*,[31] in 24 cases of macrofollicular lymphoma included in their series of 215 cases of lymphosarcoma, found a 46 per cent 5-year survival rate and an average duration of 4 5/12 years. Murray and Broders[6] found that the survival rate for their lymphosarcoma grade I was about the same as for macrofollicular lymphoma, and obtained a figure of 40.9 per cent for 5-year survivals for the combined group.

The only cures in the study series were among the 38 patients whose disease was localized when they were first seen. Of these, 18 developed generalization, 3 had local recurrence only, and 16 remained free from disease. The remaining case is too recent for follow-up. Local recurrence, in the 3 instances, developed 7/12 year, 1 year, and 10 years, respectively, after the initial biopsy. The first patient was well 1 2/12 years later, but the second and third died 1 7/12 years and 3 years later, apparently from other causes.

Thus there were 16 patients (Table VII) who remained free from recurrence (4 have died but from causes other than lymphoma). Recurrence or generalization, when it occurs, almost invariably does so within a few months of the initial biopsy, so that a "cure" may well be expected in the 6 patients who have survived 7 years or more. The prognosis must still be somewhat guarded, however, bearing in mind the example already cited in which the course of the disease was remarkably protracted. This patient had a swelling in the groin for 17 years and, following excision, there was an interval of 10 years before local recurrence developed. A second excision was performed and the patient died 3 years later at the age of 68, but unfortunately the cause of death could not be ascertained. Five months prior to death, however, careful clinical examination had failed to reveal further evidence of lymphoma.

It is interesting to note that in these surviving cases the site of the tumor was tonsil in 2 instances and lymph nodes related to salivary glands in 6 cases. It must be emphasized that the tumor was distinct from salivary gland tissue and merely mimicked a tumor of this structure. Minimal infiltration was noted, in fact, in 2 instances, but there could be no confusion with other follicular lymphoid conditions which diffusely infiltrate the salivary gland tissue.

Insufficient clinical data is available to know exactly how many nodes were involved in each instance or how many were excised, but the lesion apparently was restricted to a single node in some.

I thus come to the conclusion that a certain proportion of the patients with localized disease are cured, and the rest experience spread to other sites. Baggenstoss and Heck[1] stated that in their experience and from a review of the literature no permanent cures have been reported. It would appear, however, that provided the disease is

TABLE VII

Cases of Localized Macrofollicular Lymphoma with no Recurrence after Initial Treatment

Case	Site	Survival
		yrs.
1	Tonsil	16 8/12, alive
2	Parotid region	14 4/12, died
3	Neck	9 10/12, alive
4	Submaxillary region	8 3/12, alive
5	Tonsil	8, died
6	Neck	7 4/12, died
7	Epitrochlear gland	4 8/12, alive
8	Submaxillary region	4 3/12, alive
9	Parotid region	3 11/12, alive
10	Parotid region	3 10/12, alive
11	Groin	3 8/12, alive
12	Axilla	3 7/12, alive
13	Neck	1 7/12, alive
14	Parotid region	8/12, alive
15	Axilla	4/12, alive
16	Neck	3/12, died*

* Necropsy failed to show residual tumor tissue.

restricted to one site in a peripheral node, a small proportion of all forms of malignant lymphoma, whether macrofollicular lymphoma or not, are cured by surgery or radiation. Holmes and Schulz[32] reported 15 such patients treated by radiation and alive without recurrence for 5 years or more. One case, macrofollicular in type with involvement of inguinal lymph nodes, had survived 8 7/12 years. Gall[33] listed 20 "cured" patients with malignant lymphoma treated by radical surgery. Five had local recurrence, otherwise all had been free from disease for an average postoperative duration of 8 years, or mean total duration of 9 years. Three cases were of follicular type with nodes involving the inguinal region in 2, and neck in one. These patients had survived 4.5, 8.5, and 7.5 years, respectively, without recurrence of disease. Hell-

wig[34] considered 17.2 per cent of his series of 130 patients with malignant lymphoma apparently cured. Shimkin *et al.*[31] found that 10 patients of their 215 with various forms of lymphosarcoma remained free from clinical evidence of disease for 5 to 21 years, although in only 2 was the tumor of lymph-nodal origin. The others were of internal organs.

Gall *et al.*[3] endeavored to divide their 63 cases of macrofollicular lymphoma into four stages, on the grounds of differences in the follicular nodules. They considered that the stages could represent progressive phases in the development of the tumor, and that the groups represented fairly distinct histologic entities. Attempts to subdivide our group of cases of macrofollicular lymphoma proved to be impossible. Some of the best differentiated tumors had a short survival time and vice versa, and, more important, different stages could often be found in the same lymph node or in nodes removed at the same time, or at a previous or later date, without necessarily any logical progression. The case reported by Cohen and Bergstrom,[25] in which the follicular pattern persisted throughout the course of the disease and was widespread at necropsy, is interesting in that the total duration of the disease was only 1 year.

Summary and Conclusions

Macrofollicular lymphoma, on the basis of 136 cases, with few possible exceptions is a malignant tumor from its onset.

Sometimes the distinction from hyperplastic lymphoid tissue is difficult. Number, size, shape, marginal zonation, and phagocytic activity of the follicles have been given consideration.

Macrofollicular lymphoma usually changes during its course to diffuse forms of lymphoma.

Macrofollicular lymphoma frequently is associated with diffuse lymphoma.

The over-all prognosis is not as good as previous reports would suggest. Five-year survival was 36.1 per cent. The patient is considered probably "cured" in 8.3 per cent, all in this group being examples of localized disease.

My thanks are due to Dr. Shields Warren and Dr. Olive Gates for their help, advice, and interest in this work.

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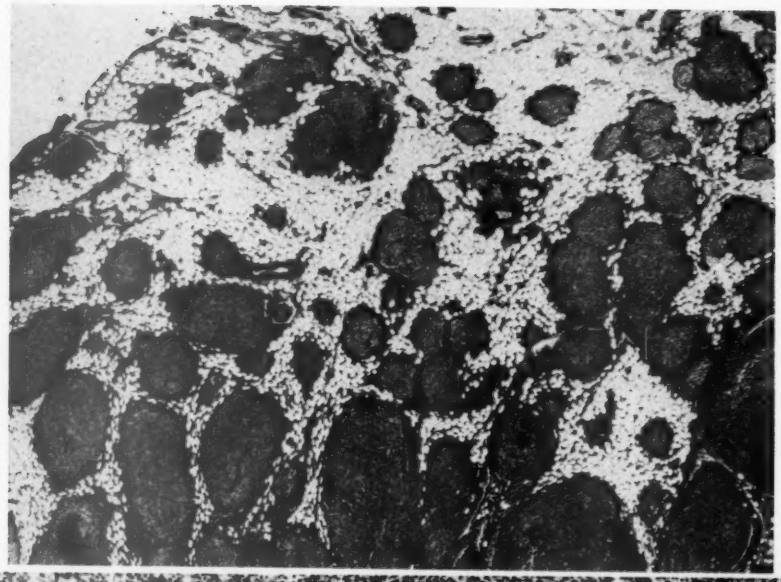
[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Adipose tissue showing follicles of macrofollicular lymphoma, some apparently quite separate. × 23.

FIG. 2. Case 21 (Table V), 1953 biopsy. Typical macrofollicular lymphoma. Follicles show peripheral rim of packed, small lymphocytes. × 125.



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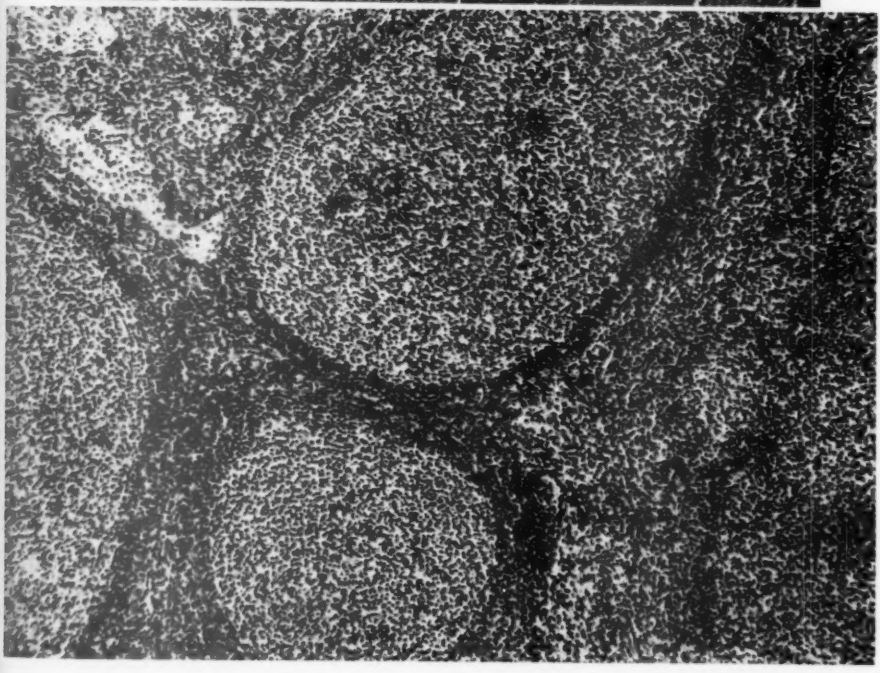

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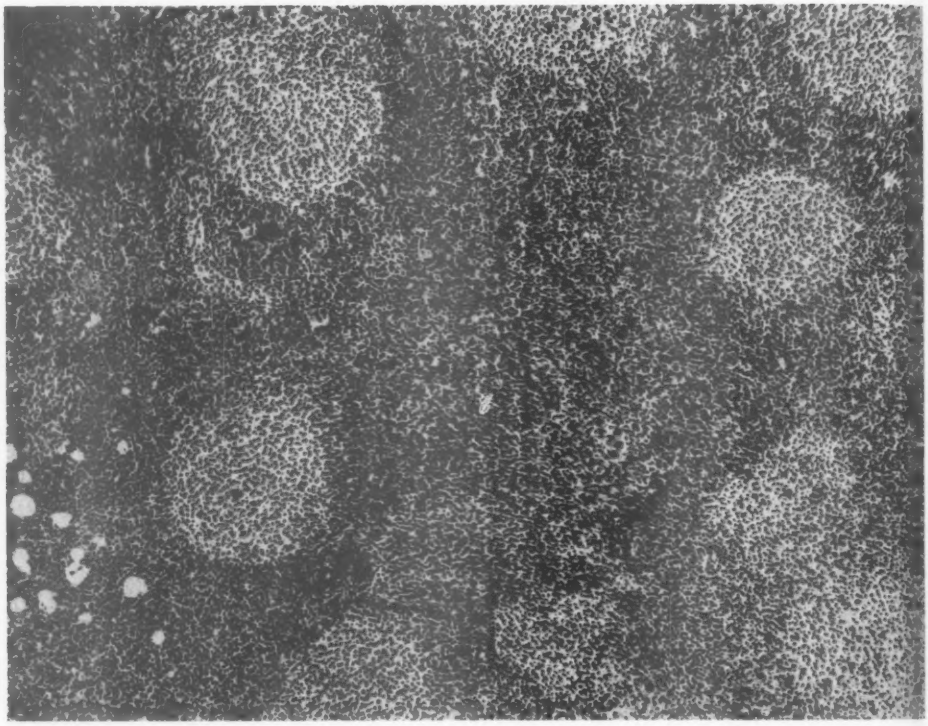
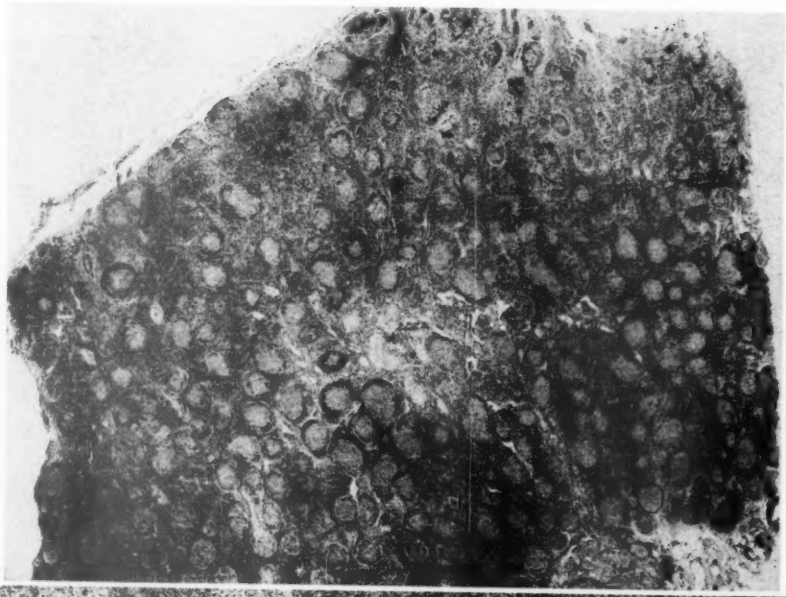


FIG. 3. Case 21 (Table V), 1950 biopsy (same case as Fig. 1). Diffuse lymphosarcoma with follicles which are widely separated. × 125.

FIG. 4. Macrofollicular lymphoma in which the follicles are smaller than usual and numerous. Death occurred 1 year after biopsy. × 28.

FIG. 5. Macrofollicular lymphoma in which some follicles assumed peculiar configurations. × 160.



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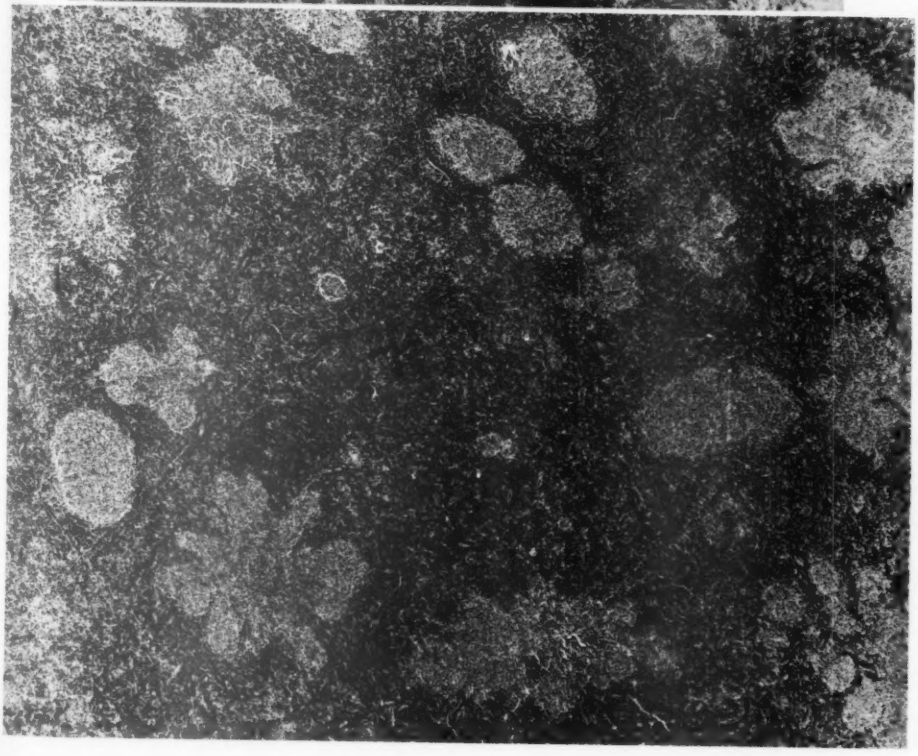

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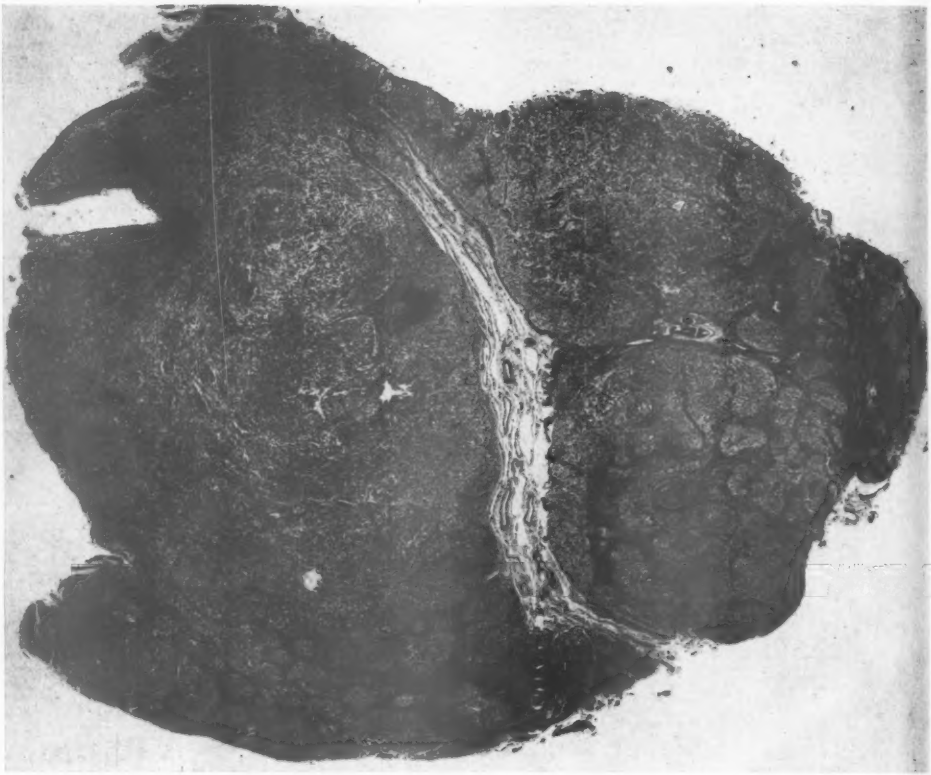
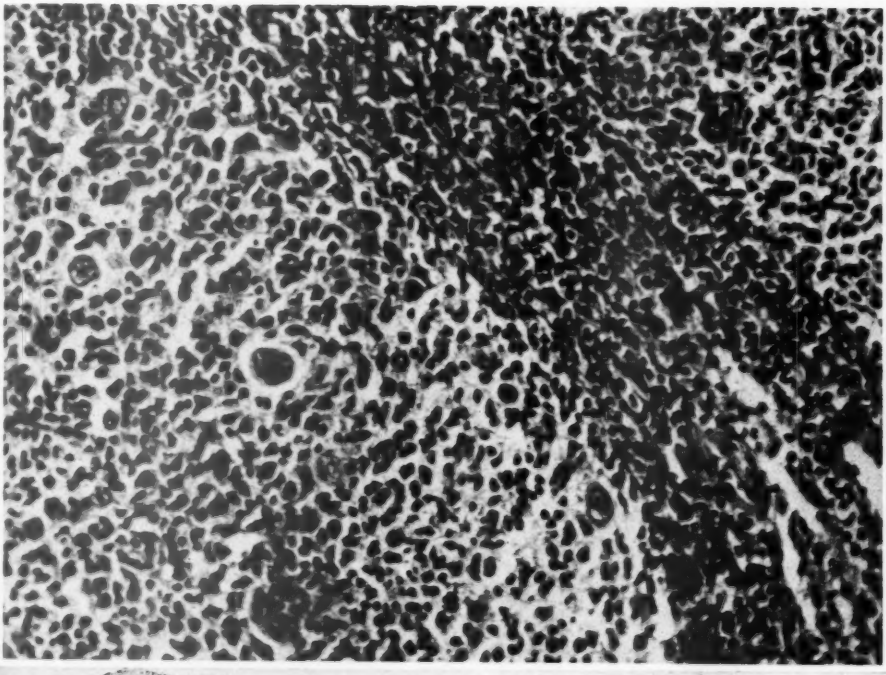


FIG. 6. Necropsy case 1 (Table IV). Macrofollicular lymphoma with confluence of follicles forming lymphoblastic lymphosarcoma distinct from the interfollicular lymphocytic tumor. × 11.

FIG. 7. Case 21 (Table V), 1955 biopsy (same case as Figs. 1 and 3). Edge of follicle (at lower left) showing occasional giant cells of Hodgkin's type. × 325.

FIG. 8. Lymph node partly disorganized by macrofollicular lymphoma with early neoplastic changes in some of the existing follicles of the other part. × 27.



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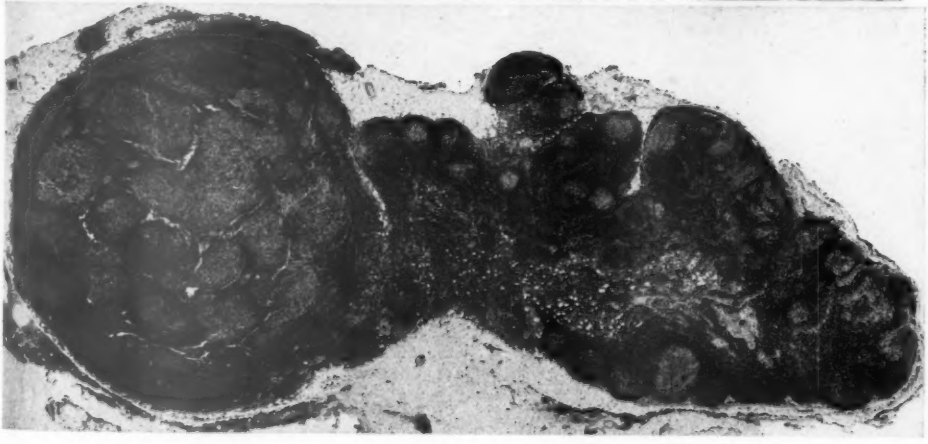

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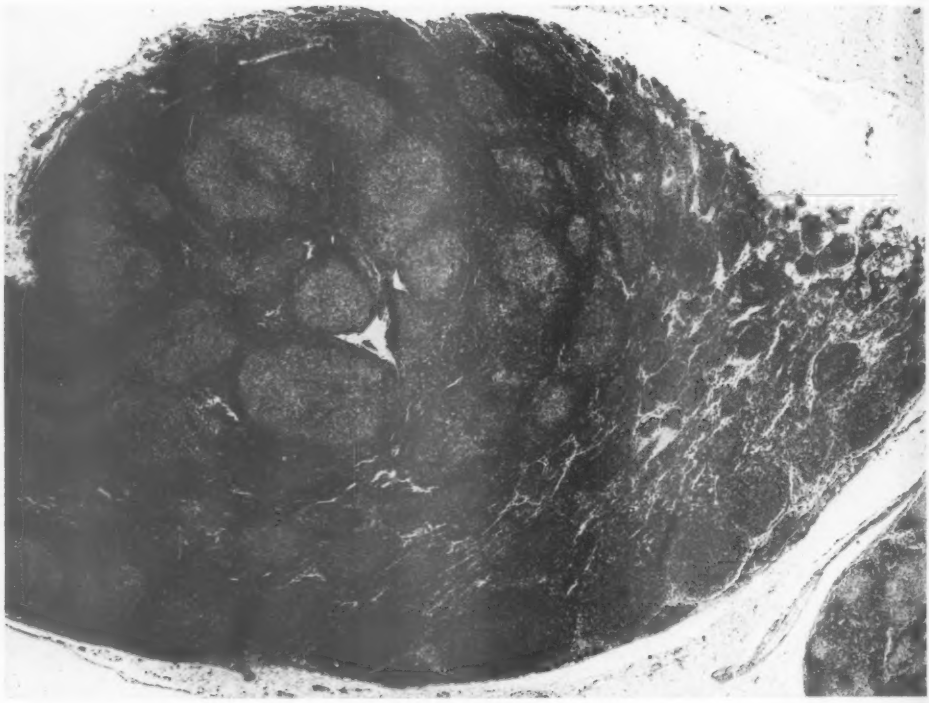
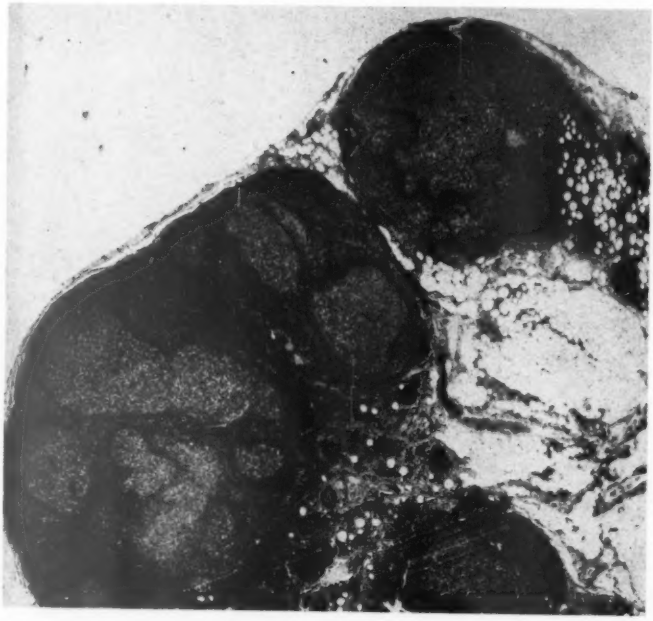


FIG. 9. Lymph node partly disorganized by macrofollicular lymphoma, with early involvement of some of the existing peripheral follicles. × 33.

FIG. 10. Hyperplastic axillary lymph node with much enlarged, irregularly shaped follicles. From radical mastectomy for carcinoma. × 35.

FIG. 11. Hyperplastic distorted follicle showing phagocytic macrophages. × 128.



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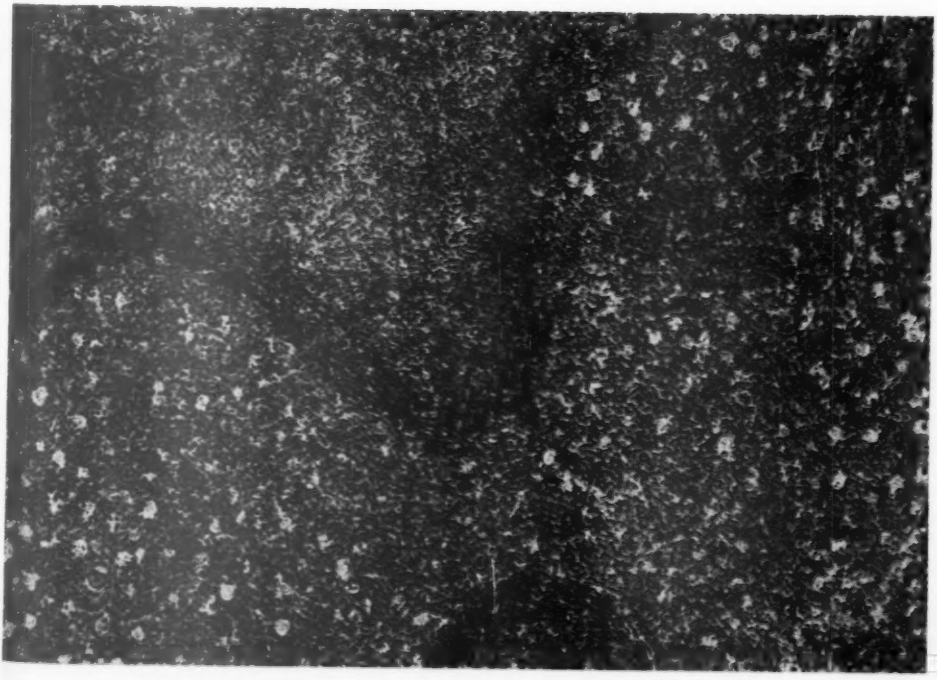

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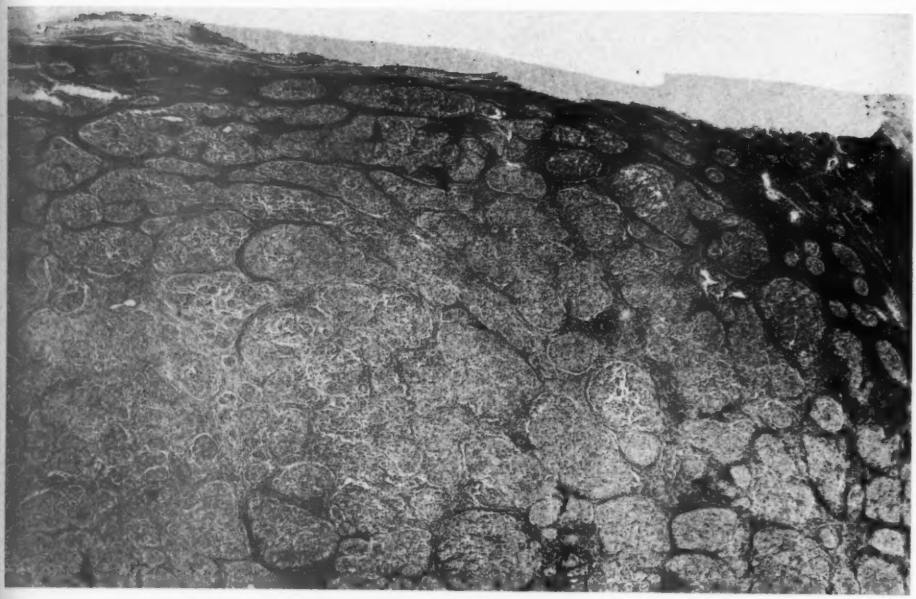
FIG. 12. Case 4 (Table VII). Lymph-nodal macrofollicular lymphoma localized to submaxillary region. Patient alive and well 8 3/12 years after excision. × 11.

FIG. 13. "Benign lymphoma of rectum" showing macrofollicular structure. Patient alive and well 16 years after local excision. × 8.

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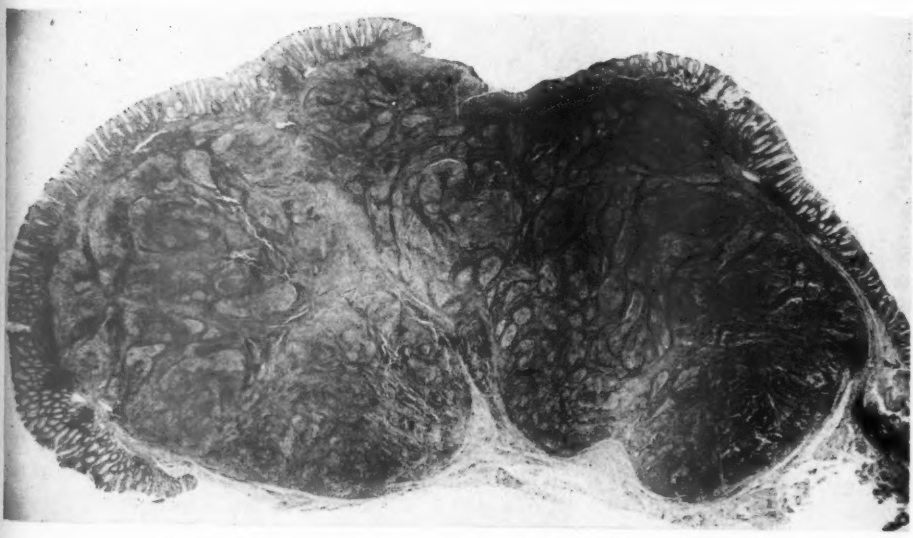

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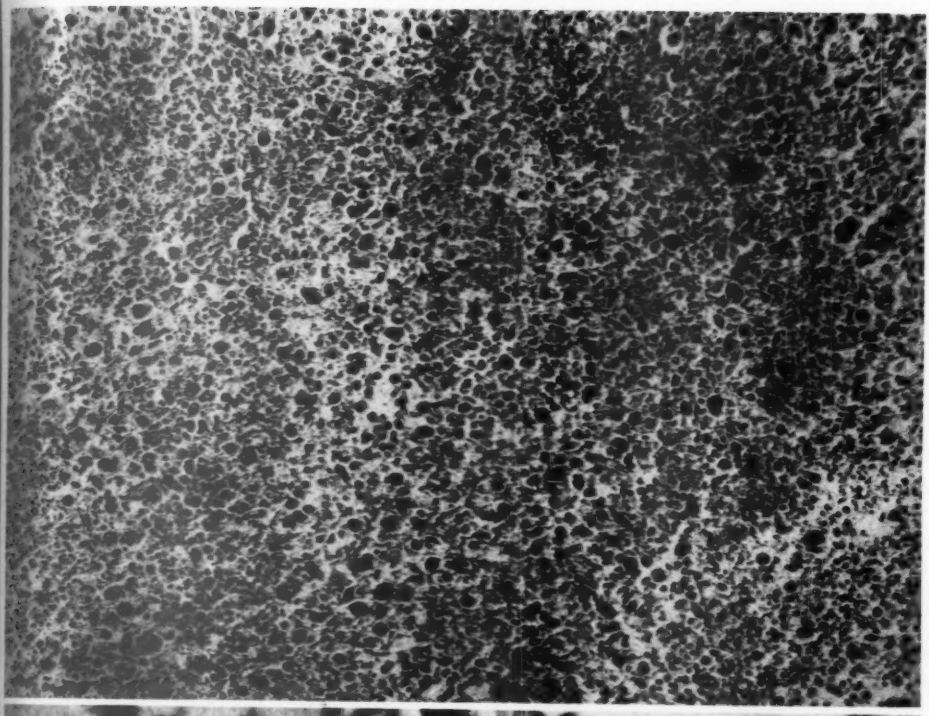
FIG. 14. Anaplastic polymorphous cell sarcoma with numerous giant cells. × 117.

FIG. 15. Higher magnification of Figure 14 to show the bizarre character of the cells. × 808.

FIG. 14. Anaplastic polymorphous cell sarcoma with numerous giant cells. × 117.

FIG. 15. Higher magnification of Figure 14 to show the bizarre character of the cells. × 808.



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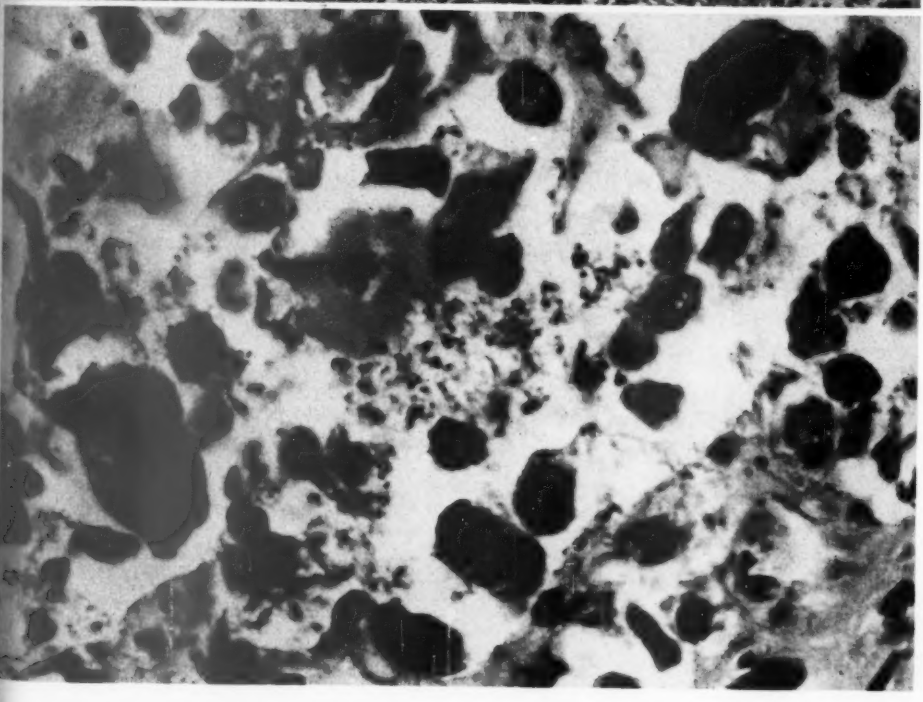

15

GASTRIC LESIONS IN HODGKIN'S DISEASE AND LEUKEMIA *

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In leukemia and Hodgkin's disease the stomach usually shows no gross lesions. However, in a few instances characteristic infiltrations occur and should be recognized.[1] These unusual changes comprise the subject of this paper and the material for this study was derived from necropsies in which the findings usually supported the clinical diagnosis of one of these lymphomas. The term Hodgkin's disease includes both Hodgkin's granuloma and Hodgkin's sarcoma. One hundred and nine cases were utilized: 45 cases of Hodgkin's disease and 64 of leukemia. Of these, 16 showed obvious gastric lesions.

Hodgkin's Disease

Brief notes on the 9 cases of Hodgkin's disease follow.

Case 1

E. S., a white man, 70 years of age, entered the hospital following a cerebral accident, but with a history of hematemesis and tarry stools. The clinical diagnosis on admission was carcinoma of the stomach. The red blood cell count was 2,600,000; the white blood cell count, 17,000 with a normal differential. After three episodes of vomiting, death followed severe hematemesis 2 days after admission.

Necropsy findings were not unusual except in the gastro-intestinal tract, which contained a large amount of blood. There was a dilated, thick-walled stomach with fourteen large, discrete ulcers (Fig. 1). A similar ulcer was seen in the duodenum and another in the proximal end of the jejunum. The spleen was enlarged and weighed 600 gm. No other gross lesions were found. Even the abdominal lymph nodes were not enlarged. Microscopic examination of the gastric wall and of the ulcers revealed a pleomorphic cellular infiltration in the mucosa and submucosa with many typical Reed-Sternberg giant cells associated with granulomatous foci in the lymph nodes, bone marrow, heart, spleen, and liver. The pathologic diagnosis was Hodgkin's disease with multicentric ulcerating Hodgkin's sarcoma of the stomach.

Case 2

O. B., a white man, 82 years old, complained of epigastric pain and anorexia for 4 months with a weight loss of 50 lbs. A Virchow's lymph node on the left and the inguinal lymph nodes on the right were palpable. There was a large palpable epigas-

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tric mass. The radiologic diagnosis was carcinoma of the stomach involving the entire stomach wall. The blood count was normal. The temperature rose to 100° F. and coma developed shortly before death.

At necropsy, the supraclavicular lymph nodes on the left were much enlarged, soft, and friable. The large, thick-walled stomach had huge encephaloid rugae (Fig. 2). The retroperitoneal, omental, and mesenteric lymph nodes were greatly enlarged. The liver was small but the hepatic hilar lymph nodes were large. The spleen also was small and appeared normal. Microscopic examination of the lymph nodes and of the stomach wall showed the typical findings of Hodgkin's sarcoma. The spleen and lymph glands appeared to be normal.

Case 3

F. M., was a white male, 20 years of age, who developed large cervical lymph nodes 5 years before his death. At that time a biopsy was reported as "Hodgkin's disease." This was followed by x-ray treatment with apparent relief for 2 years, after which a right cervical lymph node was enlarged and a second biopsy was reported as Hodgkin's sarcoma. Further x-ray treatment gave only temporary relief and the patient became progressively worse with increasing dyspnea and dysphagia. The red blood cell count was 3,300,000 and the hemoglobin, 46 per cent. The white blood cell count was 2,500 with 97 per cent polymorphonuclear leukocytes.

At necropsy, only one enlarged superficial lymph node was seen. It was in the right submaxillary area and measured 3 cm. in diameter. The peritoneal cavity contained 4,000 cc. of clear serous fluid. Granulomatous lesions were found in the lungs, liver, and spleen. The left adrenal gland was largely replaced by granulomatous tissue. The pancreas also was involved. The greater curvature of the stomach was the site of an ulcerating tumor mass, 4.5 cm. in diameter, adherent to and eroding the spleen. The surrounding gastric wall was infiltrated with granulomatous tissue. The remainder of the gastro-intestinal tract was negative. There was marked enlargement of the retroperitoneal lymph nodes. Microscopic examination showed granulomatous tissue of typical Hodgkin's type in the lymph nodes, spleen, liver, lungs, and ulcerating gastric tumor mass.

Case 4

F. W., a white male, 37 years old, had enlarged lymph nodes in the right axilla with diagnosis by biopsy of possible Hodgkin's disease. X-ray therapy was followed by disappearance of the enlarged nodes. Two months later, sternal pain, dyspnea, and epigastric discomfort developed, associated with enlarging cervical lymph nodes. Additional x-ray treatment gave no improvement. The red blood cell count was 3,800,000; the white blood cell count, 8,100 with 82 per cent polymorphonuclear leukocytes.

At necropsy, the cervical, inguinal, mediastinal, and hilar lymph nodes were large and discrete. Focal cellular infiltrations were seen in the liver and spleen. Nodular lesions also were found in the mucosa

of the stomach, with localized enlargement of some of the rugae. Microscopic examination showed typical Hodgkin's granulomatous lesions in the lymph nodes, liver, spleen, kidneys, pancreas, and in the gastric nodules.

Case 5

R. B. was a white male, 50 years of age, who had a "cyst" removed from the groin, with a diagnosis of Hodgkin's disease. There was enlargement of the cervical lymph nodes. In spite of x-ray therapy, the patient became worse and returned to the hospital 6 months later with increased abdominal pain. The red blood cell count was 3,720,000; hemoglobin, 78 per cent; and the white blood cell count, 3,300. Death occurred 3 months later.

At necropsy, the cervical, inguinal, and epitrochlear lymph glands, as well as those in the retroperitoneal and mesenteric regions, were greatly enlarged and associated with nodular infiltrations of many of the gastric rugae (Fig. 3). Some of these nodules in the stomach showed early ulceration. Microscopic examination presented the typical granulomatous proliferation of Hodgkin's disease in the enlarged lymph nodes, in the spleen, and in the nodular infiltrations in the gastric rugae.

Case 6

C. H. was a white man, 82 years old, who complained of anorexia, weight loss, and weakness of 3 weeks' duration. A large, palpable mass was felt in the abdomen. There were bilateral inguinal hernias. His temperature was 103° F. Death occurred 1 hour after admission to the hospital.

Necropsy revealed large, discrete lymph nodes in both axillae and in the retrosternal area. All abdominal lymph nodes were markedly enlarged, and there was a mass of discrete nodes at the root of the mesentery. A discoid, ulcerating, solitary nodule, 4 cm. in diameter (Fig. 4), was found in the mucosa of the lower anterior surface of the stomach. The spleen was enlarged, weighing 1,050 gm., and the cut surface showed scattered, small, white nodules 1 to 3 mm. in diameter. Microscopic examination showed granulomatous proliferation, typical of Hodgkin's disease, in all lymph nodes, the liver and spleen, and in the ulcerating mass of the stomach wall.

Case 7

C. D. was a white male, 47 years of age, who had a painless right cervical lump, with pain in the right side of the face, head, and in the abdomen, and cachexia. Tissue taken for biopsy of an ulcer of the pharynx was reported as lymphosarcoma. There was bilateral enlargement of the cervical and axillary lymph nodes. There was also considerable dysphagia, weakness, and weight loss. The white blood cell count was 10,000 with 91 per cent polymorphonuclear leukocytes.

Necropsy showed enlarged cervical, axillary, mediastinal, and retroperitoneal lymph nodes. Both adrenal glands were enclosed by indurated cellular tissue associated with a thick, ulcerated lesion (Fig. 5)

along the greater curvature of the stomach. There was some infiltration in the adjacent rugae. The only other lesion was a dense, ulcerated mass in the jejunum, 50 cm. below the pyloric ring. Microscopic changes typical of Hodgkin's granuloma were seen in all of the enlarged lymph nodes, in the tissue surrounding the adrenal glands, and in the ulcerated masses in both the stomach and jejunum.

Case 8

W. H. was a while male, 71 years of age, with a clinical diagnosis of Hodgkin's disease and enlarged inguinal and axillary lymph nodes. There had been repeated x-ray treatments. Râles were heard in both lung fields. The red blood cell count varied from 2,900,000 to 3,600,000; white blood cell count, from 1,150 to 5,000 with a normal differential count. There were about 68 per cent polymorphonuclear leukocytes. Hemoglobin varied from about 58 to 61 per cent. Nitrogen mustard treatment was given with no improvement.

At necropsy, 2,000 cc. of bloody fluid was found in each pleural cavity. There was marked enlargement of the axillary and inguinal lymph nodes. The patient was markedly emaciated. There were ulcerated nodules in the gastric mucosa and in the intestines, and considerable infiltration of the cardiac end of the stomach with some ulceration. Retroperitoneal, mesenteric, and mediastinal lymph glands were very large. The renal capsules seemed to be infiltrated, and cellular nodules were seen in the left renal pelvis. Bilateral areas of induration were felt in the lungs and some nodules were seen in the liver. Microscopic examination of the lesions in the lymph glands, the stomach, and the lungs showed typical granulomatous proliferation of Hodgkin's disease. The same lesions, associated with many Dorothy Reed giant cells, were seen in the lymph nodes, liver, and kidney. The spleen, however, appeared to be normal.

Case 9

C. P., white male, 31 years of age, was ill with malaise and fever for 20 months before death. Two months after the onset, the cervical lymph nodes became swollen, associated with fever, cough, and loss of weight. Seven months later biopsy of a cervical lymph node revealed Hodgkin's disease. X-ray treatment brought no improvement. The patient was admitted to the hospital 2 months before his death. At that time general lymphadenopathy was noted. Red blood cell count was 3,830,000; white blood cell count, 5,100, with 91 per cent polymorphonuclear leukocytes. The temperature varied from 101° to 103° F. Multiple subcutaneous nodules appeared a few weeks before death. Nitrogen mustard administration was of no avail. There was considerable icterus. The patient died following a fall from a hospital window.

At necropsy, there was a widespread enlargement of all lymph nodes, infiltration of the lungs, and multiple gastric nodules with ulceration and hemorrhage. Subcutaneous nodules also were present. There were many granulomatous nodules in the liver, especially around the hilum and along the course of the larger bile ducts. Microscopically, the

granulomatous foci showed the pleomorphism, fibrosis, eosinophilic cells, multilobulated nuclei, and giant cells usually seen in Hodgkin's disease. These changes were seen also in the liver, spleen, lungs, lymph nodes, subcutaneous nodes, and in the gastric lesions.

SUMMARY OF GASTRIC LESIONS IN CASES OF HODGKIN'S DISEASE

In none of our cases of Hodgkin's disease were the gastric lesions the only evidence of disease. In case 1 the gross findings at post-mortem examination indicated a primary process in the stomach, but microscopic studies revealed granulomatous lesions in the spleen, liver, bone marrow, and heart, indicating a generalized process with the gastric lesion predominating. In 3 of our cases single, large, ulcerative infiltrations of the gastric mucosa were seen (Figs. 4 and 5). In 3 instances multiple, ulcerating infiltrations of the stomach were striking, especially so in case 1 (Fig. 1). In case 2 there was extensive gastric infiltration with formation of large encephaloid rugae and superficial ulceration (Fig. 2). Two of our cases showed nodular infiltrations with superficial ulceration (Fig. 3). In most of our cases the main site of the infiltration was in lymph nodes, especially those of the abdominal group.

LEUKEMIC LYMPHOBLASTOMA

There were also 64 cases of leukemia available for study. Of these, 7 showed distinct gastric lesions. A brief note on each follows.

Case 1

James T. was a white male, 71 years old, who complained of difficult urination, nocturia, and loss of weight over a period of 1 year. Physical examination showed very large cervical and axillary lymph nodes. White blood cell count was 20,000 with 77 per cent lymphocytes. Temperature was 101° to 102° F. The prostate was large and firm. Several large calculi were found in the gallbladder. The liver was also considerably enlarged. The patient had advanced hypertrophic arthritis. The spleen was easily palpable. The clinical diagnosis was lymphatic leukemia.

At necropsy, all lymph nodes were greatly enlarged. The liver also was enlarged. It weighed 2,350 gm., and there was a small subcapsular hemorrhage. The spleen was large, soft, and weighed 720 gm. The large, dilated heart weighed 485 gm. Both kidneys were large and showed extensive areas of cellular infiltration. The gastric wall was diffusely thickened and the rugae were unusually large and encephaloid (Fig. 6), but the antrum was not much involved. Microscopic studies showed widespread myeloid infiltration in the stroma and mucosa of the heart, liver, spleen, kidneys, and appendix, and in the wall of the stomach, in which most of the myeloid infiltration was in the mucosa and submucosa. The giant rugae were strikingly prominent but showed no ulceration.

Case 2

John T. was a white male, 60 years of age, who complained of loss of weight, anorexia, and weakness. Two years previously he had had "walking pneumonia." This was followed by enlargement of the cervical lymph nodes, biopsy of one of which was reported as showing leukemia. The white blood cell count was 7,000 with 42 per cent polymorphonuclear leukocytes, 21 per cent lymphocytes, and 21 per cent myelocytes. The sternal bone marrow revealed atypical cells and many mitotic figures, suggestive of acute lymphatic leukemia. A clinical diagnosis of acute lymphatic leukemia was made on the basis of the bone marrow smears. The patient became rapidly worse while in the hospital. Bone pains became prominent and low-grade fever and night sweats developed.

At necropsy, the infraclavicular, retroperitoneal, and mesenteric lymph nodes were very large, soft, and friable. The liver weighed 1,830 gm. and showed extensive periportal cellular infiltration. Similar cellular infiltration was prominent also in the spleen, heart, kidneys, lung, and bone marrow. The gastric wall was diffusely thickened with production of giant encephaloid rugae. The antrum was not involved. Microscopically, the same myeloid infiltration noted in the other organs extended into the mucosa and submucosa of the gastric wall.

Case 3

G. H. was a white female, 70 years old, who complained of loss of weight, fever, and enlarged cervical lymph nodes. Her blood pressure was 120/80 mm. of Hg and she was somewhat cyanotic. The liver and spleen were both easily palpable. The white blood cell count was 5,000 with 42 per cent lymphocytes and 56 per cent polymorphonuclear leukocytes. A lymph node biopsy was reported as showing leukemia. The temperature rose to 103° F. X-ray therapy gave no help. A streptococcus was isolated from a blood culture. A clinical diagnosis of aleukemic leukemia was made.

At necropsy, there was a marked enlargement of the cervical, axillary, retroperitoneal, and mesenteric lymph nodes. The stomach wall was thickened and giant encephaloid rugae were seen. Microscopically, there was extensive cellular infiltration in the stroma of the heart, liver, and lymph nodes, and in the mucosa and submucosa of the stomach.

Case 4

W. P. S. was a white man, 65 years of age, who complained of weakness, dyspnea, and malaise for 2 months. There also had been anorexia and loss of weight in the past 6 months. There was severe pallor and enlarged inguinal and axillary lymph nodes were found. The red blood cell count was 2,700,000; the white blood cell count, 17,100 with 56 per cent lymphocytes. Cutaneous and rectal hemorrhages were prominent. The clinical diagnosis was subacute lymphatic leukemia.

At necropsy, there was an extensive enlargement of the abdominal as well as of the inguinal and axillary lymph nodes. The liver weighed 2,100 gm. and there was marked cellular infiltration of the portal spaces. The stomach showed many discrete, nodular, cellular infiltrations, some of the larger of which presented early ulceration (Fig. 7).

A similar leukemic infiltration was seen in the bone marrow, kidneys, spleen, heart, lymph glands, and gastric mucosa.

Case 5

E. J., a Negress, 23 years old, had been treated for severe anemia, subconjunctival hemorrhages, and chest pain. She was 7 months' pregnant when admitted to the hospital. The white blood cell count was 17,600 with 90 per cent lymphocytes, and the red blood cell count was 1,000,000. A clinical diagnosis of acute leukemia was made. The hospital course was rapidly downhill in spite of therapy and blood transfusions. A cesarean section with the removal of a live child was done 3 days before death.

At necropsy, the axillary and abdominal lymph nodes were much enlarged. There was 2,000 cc. of bloody fluid in the peritoneal cavity. The stomach wall was thickened focally and many of the rugae were enlarged and encephaloid. Microscopically, there was extensive cellular infiltration throughout the gastric mucosa and submucosa. There were similar infiltrations in the liver, kidneys, spleen, and lymph nodes. The microscopic picture was typical of leukemia.

Case 6

R. W. was a white man, 68 years old, with a 5-year history of monocytic leukemia. His chief complaints were pain in the back and shoulders, weakness, and vomiting. There was extensive swelling of all superficial lymph nodes. The red blood cell count was 2,700,000; the white blood cell count, 200,000 with 91 per cent monocytes and 4 per cent polymorphonuclear leukocytes. The hospital course was rapidly downhill, death occurring rather suddenly.

At necropsy, there were many cutaneous, submucous, and subserous hemorrhages. All lymph nodes were enlarged, discrete, and friable. The liver and spleen were enlarged, weighing 2,160 and 325 gm., respectively. The gastric rugae often were swollen and showed many superficial ulcers, the largest of which measured 28 mm. in diameter. The walls of these ulcers showed infiltration with many immature blood cells which were present also in the stroma of the liver, heart, kidneys, spleen, and lymph nodes.

Case 7

G. W. was a white woman, 81 years of age, who complained of weakness, loss of weight, abdominal pain, and vomiting. Physical examination showed abdominal distention and a mass which was not identified roentgenologically. Red blood cell count was 2,650,000; white blood cell count, 7,500 with 83 per cent polymorphonuclear leukocytes. Hemoglobin was 53 per cent. She also had a fibrillating heart, as shown by an impaired electrocardiogram. She was discharged after a 3 weeks' stay in the hospital with a diagnosis of anemia and heart disease. She was admitted 2 months later with blood pressure of 96/48 mm. of Hg and a temperature of 99.6° F. She developed pulmonary edema and died a few hours later.

Necropsy showed a large spleen, weighing 1,273 gm., large masses of discrete, swollen, retroperitoneal and mesenteric lymph nodes, and a

liter of fluid in each pleural cavity. All abdominal lymph nodes were enlarged, soft, and friable. The peripheral lymph nodes, however, were not particularly prominent. The gastric mucosa showed nodular infiltration with superficial erosions. Bone marrow smears made at necropsy showed a marked accumulation of immature blood cells. This finding was regarded by the hematologist as indicative of leukemia. Typical immature blood cell infiltration was seen in the abdominal lymph glands, the spleen, the liver, and the nodules in the gastric mucosa.

SUMMARY OF GASTRIC LESIONS IN CASES OF LEUKEMIC LYMPHOBLASTOMA

The most striking lesion in 7 cases of leukemia was the extensive cellular infiltration in the gastric mucosa and submucosa. In case 1 the infiltration was diffuse and widespread throughout the entire stomach wall with formation of giant rugae resembling cerebral convolutions and called by some authors[2-6] encephaloid rugae (Fig. 6). In 3 of the cases the gastric infiltrations were more localized and in places almost nodular. In one instance (case 4) the nodular character was most striking, with frequent ulceration and umbilication (Fig. 7). Of the 7 cases, ulceration was present in 3. All 7 cases were of the lymphatic type of leukemia, 2 being classed as acute leukemia. Clinically, 2 cases were regarded as atypical and classed as aleukemia leukemia. No cases of typical myeloid leukemia were noted in this group, though one was classed as a monocytic leukemia.

Discussion

Of a total of 109 necropsies in cases of Hodgkin's disease and leukemia, gastric involvement was noted in 16. Nine of our cases were of Hodgkin's disease and 7 of leukemia. Wells and Maver[5] found 7 instances of gastric involvement without concomitant intestinal lesions in 238 cases of leukemia. In the 9 cases of Hodgkin's disease, no true "primary" gastric lesion was found. While primary Hodgkin's disease of the stomach has been reported in the literature,[7-9] it is extremely rare.[10] In fact, the only case of what appears to be truly primary gastric Hodgkin's disease in which complete post-mortem study was provided is that reported by Singer.[11] Most reported cases are based on surgical material.[12-15] Portmann and others[16] collected 46 cases from the literature and added 6 of their own. In the instances in which necropsy material was available, often only a gross examination was described or the microscopic report appeared to be incomplete.[17-19] Our first case (Fig. 1) was at one time regarded as "primary"

in the stomach, but a study of microscopic slides from the liver, bone marrow, spleen, and lymph nodes revealed characteristic histologic changes of Hodgkin's disease.

Our cases illustrate lesions of the stomach of four types found in Hodgkin's disease.[16,20] First, there are multiple, flat ulcers (Fig. 1). The second form is the solitary, ulcerating tumor with or without other visceral involvement (Figs. 4 and 5). This is the form reported frequently by the surgeon, with good results following resection.[9,13,21] The third type presents multiple, small nodules with or without ulceration (Fig. 3). The fourth manifestation of gastric Hodgkin's disease is the diffuse infiltration of the entire stomach wall with the formation of encephaloid rugae (Fig. 2). The last two forms occur also with leukemia and are then indistinguishable from Hodgkin's disease except on microscopic examination.

The gastric lesions in leukemia are of two main types: the diffuse, infiltrating, encephaloid type (Fig. 6), affecting the entire stomach wall, and the nodular form (Fig. 7). Most cases present lesions intermediate between these two forms.

Leukemia and Hodgkin's disease are both systemic diseases, the former affecting the hematopoietic tissues and the latter the reticuloendothelial system. This is generally accepted for leukemia but, since the literature contains many examples of so-called primary gastric Hodgkin's disease,[15-17] there must be doubt either of its existence as a systemic disease or of the accuracy of these reports, most of which are based on surgical material.[15,18,21,22] The gastric lesions may be the first to be noted clinically in both leukemia[4] and Hodgkin's disease[12,23] and it is only later in the course of these diseases or at necropsy[7,24] that the general distribution of the lesions becomes obvious.

The gastric lesions in Hodgkin's disease tend to ulcerate. This was true in all but 2 of our cases. This tendency was not so striking in leukemia, only 3 cases of which showed ulcers. On the other hand, giant encephaloid rugae were more prominent in the leukemias. However, since diffuse infiltrations without ulceration occurred in both conditions, they could not be distinguished grossly and could be recognized only by microscopic study.[25]

The large encephaloid rugae seen with some of these lymphomas must be distinguished from primary chronic hyperplastic gastritis[26-28] and from multiple gastric polyposis,[29,30] first described by Menetrier[31] in 1888. Both conditions are relatively rare. Neither of them shows cellular infiltration in the gastric mucosa[30] or submucosa. The latter frequently become malignant but not the former.[26]

In leukemia the gastric lesions are always accompanied by changes

in bone marrow and lymph nodes. Occasionally, the gastric lesions grossly may precede those in the lymph nodes.[4] The gastric involvement is usually in the lymphatic type of leukemia[2,4] and was first noted by Briquet in 1838.[2,3,6] We have not found any instance of gastric involvement with myeloid leukemia.

The first of our cases of Hodgkin's disease was unique in the presence of fourteen large saucer-shaped ulcers without involvement of the regional lymph nodes. It was only on microscopic study that lesions of Hodgkin's disease were revealed in the regional lymph nodes, bone marrow, spleen, heart, and liver.

The presence of a single, large, ulcerating lesion in the stomachs of 3 of our 9 cases of Hodgkin's disease is of considerable interest, especially to the surgeon.[19,22,32] They were all associated with similar lesions of the abdominal lymph nodes and, as in most cases reported in the literature,[10] were mistaken clinically for carcinoma.[10,19] The prognosis following resection of such isolated ulcerating lesions is much better than for carcinoma.[16,32] Many patients have been reported alive 5 to 10 years after resection.[7,10] Berg[23] reported such a patient alive 13 years after resection. Consequently, a gastric lesion resembling carcinoma with metastases should always be biopsied and resection abandoned only after it has been proved to be a true[16,32] carcinoma. Apparently, in some instances of systemic lymphoblastoma the most striking local manifestation may be the ulcerating gastric lesion.[10,23] With removal of this major lesion, the general process may be held in abeyance for years[10] before the systemic nature of the disease is revealed.

Summary

Both Hodgkin's disease and leukemia may be associated with cellular infiltration in the gastric mucosa, with or without secondary ulceration. The gross lesions are indistinguishable although ulceration is more common and distinctive in Hodgkin's disease.

Gastric lesions in Hodgkin's disease and leukemia are not common and, when present, are manifestations of a systemic disease, and are rarely, if ever, primary in the stomach. Evidences of gastric involvement may precede manifestations of other visceral involvement but a thorough post-mortem examination with complete microscopic study almost always reveals wider dissemination.

Four types of gross gastric manifestations occur in Hodgkin's disease: (a) multicentric ulcerative lesions (Fig. 1); (b) diffuse encephaloid rugae (Fig. 2); (c) a solitary ulcerative lesion (Figs. 4 and 5); (d) nodular infiltration (Fig. 3).

Two types of gross gastric lesions occur in leukemia, often with

superficial ulceration: (a) localized nodular infiltrations (Fig. 7); (b) diffuse infiltrating changes with encephaloid rugae (Fig. 6).

The giant encephaloid rugae occasionally occurring in both of these lymphomas must be differentiated from the comparatively uncommon primary hyperplastic gastritis and the equally rare diffuse gastric polyposis. There is no atypical cellular infiltration in either of these conditions. Malignant changes frequently occur in the latter.

Since most cases of Hodgkin's disease of the stomach are mistaken for carcinoma clinically and since the prognosis of ulcerating gastric Hodgkin's disease is much better, biopsy should always be done.

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LEGENDS FOR FIGURES

Figs. 1 to 5 are from cases of Hodgkin's disease.

FIG. 1. Case 1. Gross photograph of ulcers of the stomach in Hodgkin's disease. Of note are (a) many flat or discoid ulcers in the stomach mucosa, and one in the proximal part of the duodenum; and another (b) solitary ulcer in the jejunum.

FIG. 2. Case 2. Gross photograph of stomach in Hodgkin's disease showing massive infiltration of the gastric mucosa, forming large encephaloid rugae with superficial ulceration.

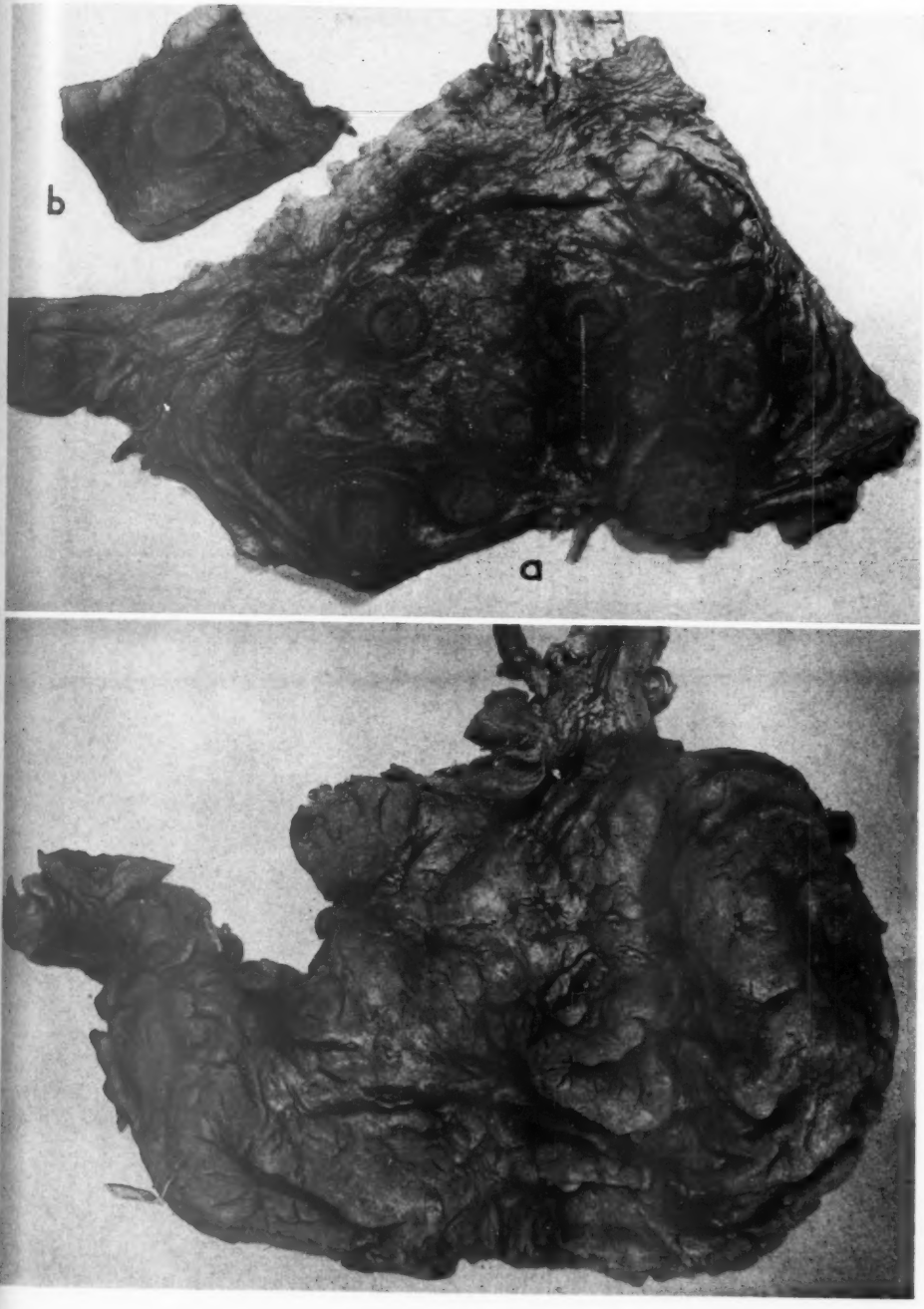

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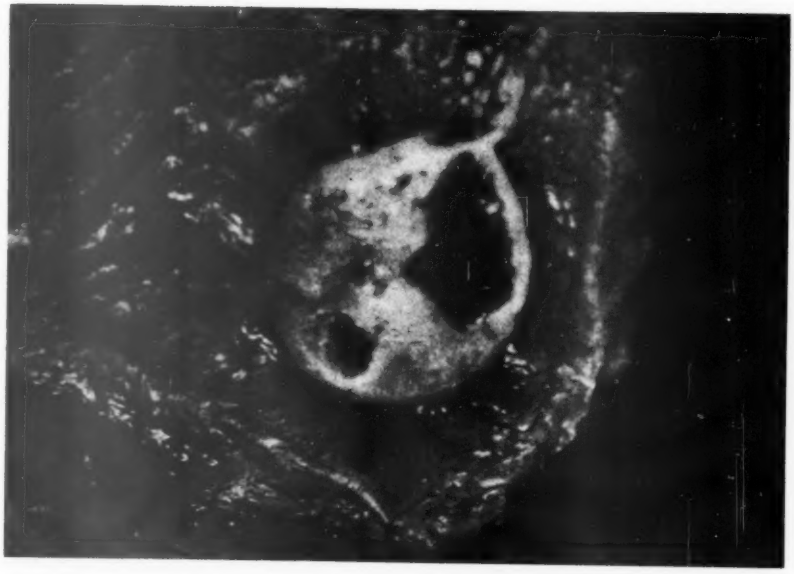
FIG. 3. Case 5. Gross photograph of gastric lesions in Hodgkin's disease with the formation of an isolated nodule (a), patchy infiltrations of the rugae (b), and an ulcerated infiltration (c).

FIG. 4. Case 6. Gross photograph of a solitary, discoid, ulcerating infiltration in the gastric mucosa in Hodgkin's disease.

FIG. 5. Case 7. Gross photograph of a solitary ulcerating infiltration in the gastric mucosa (a) and a similar lesion (b) in the jejunum in Hodgkin's disease.



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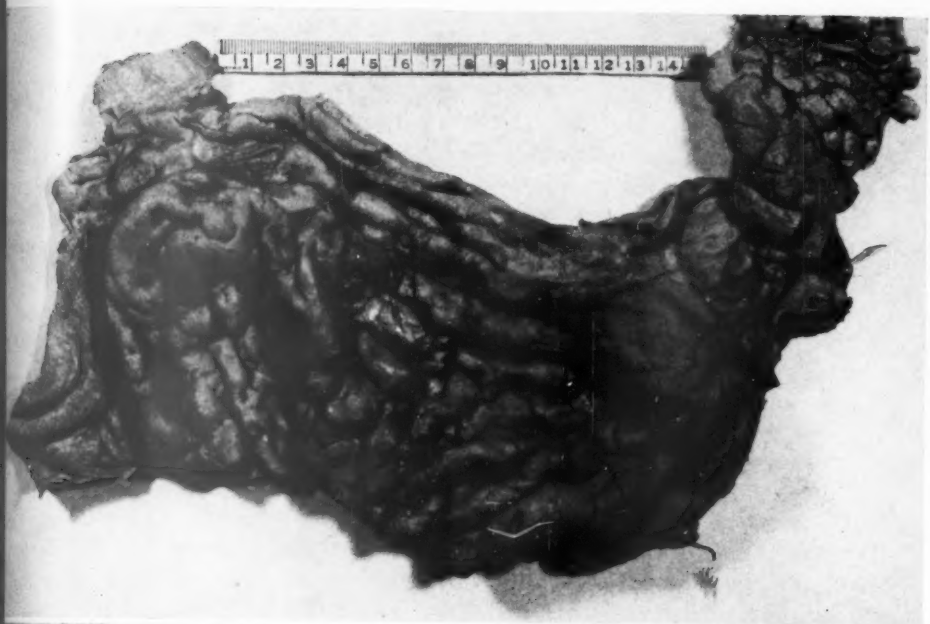
Figs. 6 and 7 are from cases of leukemia.

FIG. 6. Case 1. Gross photograph of gastric mucosa in lymphatic leukemia showing massive diffuse infiltration with the formation of large encephaloid rugae, similar to those of Figure 2 in Hodgkin's disease.

FIG. 7. Case 4. Gross photograph of gastric mucosa in lymphatic leukemia, showing many large, distinct, nodular infiltrations with beginning ulceration.



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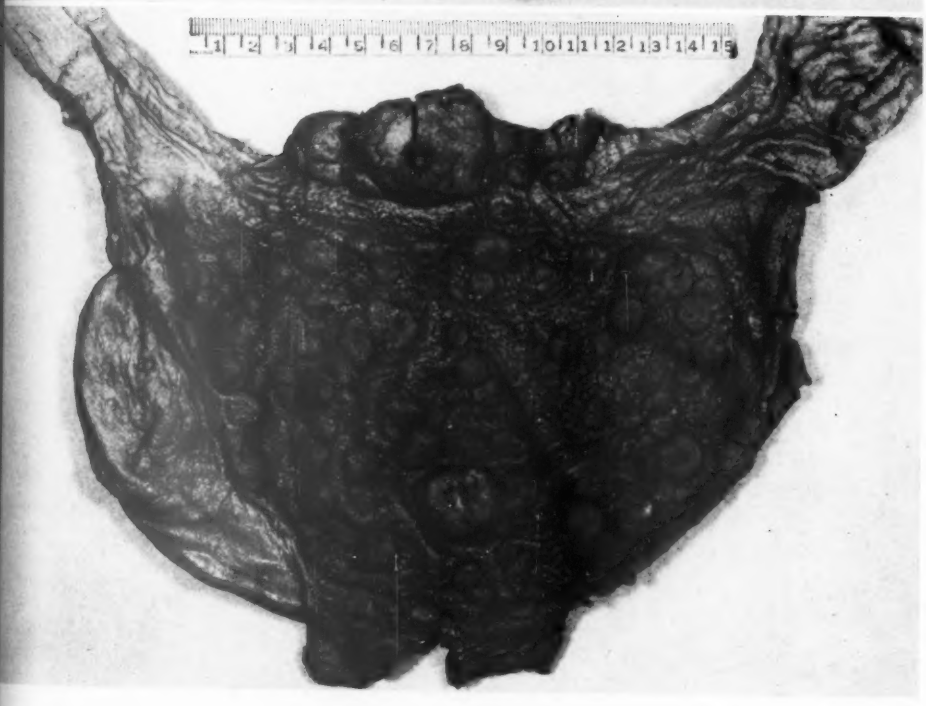

7

THE THORACIC DUCT IN MALIGNANT DISEASE *

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The thoracic duct was discovered in 1647 by Pecquet[1] and described by him in 1651. Mascagni,[2] in 1787, however, was the first to describe this structure in any detail. Its importance in the spread of malignant disease awaited the observation of Virchow,[3] in 1848, that carcinoma of the stomach sometimes was associated with enlargement of left supraclavicular lymph nodes. Troisier,[4] in 1889, noted that other abdominal cancers, as well as those of the stomach, produced enlargement of the left supraclavicular nodes.

Actual description of anatomical changes occurring with cancerous involvement of the duct seems to have begun with Cooper's[5] account in 1798. He found ductal involvement in a case of testicular neoplasm in which the cisterna and thoracic portion of the duct were dilated, thick-walled, and filled with tumor which was attached to the walls. In 1883, Enzmann[6] collected still other cases reported by Andral[7] (1824, carcinoma of the uterus), Virchow[8] (1845, carcinoma of the esophagus), and Weigert[9] (1880, carcinoma of the rectum). He added to these a case of his own of uterine carcinoma in which there was extensive ductal encroachment. He believed that involvement of the thoracic duct was rare and occurred by lymphatic permeation from adjacent draining nodes and less often by direct extension. He also emphasized the importance of obstruction of the duct by lymph thrombi, by scarring and thickening of the wall, and by pressure from a tumor mass or large tumorous lymph nodes.

Excellent reviews of the earlier literature were given by Leydhecker,[10] Winkler,[11] and Schwedenberg.[12] These workers reported detailed anatomical changes in their own cases, and Winkler emphasized the importance of retrograde spread of tumor through the duct and its lymphatics. Retrograde spread through intestinal lymphatics also was noted and emphasized by Waldeyer[13] in 1867. By retrograde injections, Schmidt-Mülheim[14] demonstrated in 1877 the incompetence of valves of lymphatics entering the cisterna. Schwedenberg believed that the thoracic duct was the main pathway by which intra-abdominal cancer reached the lungs, though actual involvement of the duct itself was considered rare.

Few studies yielding statistics are available concerning incidence

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of involvement of the thoracic duct or supraclavicular nodes. Willis,[15] in 1952, gave an incidence of 6.3 per cent involvement of the duct in sub-diaphragmatic tumors. He emphasized that involvement is not unusual and that the impression of rarity persists because of neglect of study of the duct at necropsy. Washburn,[16] in 1938, found tumorous invasion of four ducts in 50 cases of cancer, but the cervical portions of the ducts were not examined. Studies such as that by Viacava and Pack,[17] in 1944, concern only clinical findings, for, in a survey of the records of 4,365 cases of cancer, only 122, or 2.8 per cent, revealed supraclavicular metastases. As will be emphasized later, the nodes of Virchow's group are deeply placed and frequently not palpable even though they contain tumor. Whether the nodes are palpable is not a true indication of incidence of involvement.

There have been numerous reports of single cases with involvement of the thoracic duct and/or the supraclavicular node; suffice it to say that almost any tumor may involve the duct. Those arising in the stomach, uterus, lung, colon, and esophagus have been reported most often. Sarcomas, particularly the lymphomas, likewise involve the duct with some degree of regularity, and in these cases the supraclavicular nodes very frequently show tumor.

Method

In 150 consecutive cases of tumor death, excluding brain tumors and leukemia, the entire thoracic duct, its main tributaries, draining nodes, and left supraclavicular nodes were removed and dissected. Multiple blocks representing cross sections and lymph node sections were prepared for microscopic study.

The removal was carried out at necropsy before the aorta was removed but after the body cavities had been eviscerated. The duct is easily identified by separation of the tissues between the aorta and azygos vein just above the diaphragm. Once identified, this structure is then dissected distally to obtain the cisterna and its lumbar and mesenteric branches. Proximal dissection is done until the level of the mediastinal outlet is reached. At this point it is convenient to remove the aorta. If the arch is not removed, the descending portion is amputated and the arch dissected and turned upward along with its branches. This allows removal of the cervical portion of the duct and the attached supraclavicular nodes. The duct passes beneath the left carotid artery. Usually no attempt is made to carry out minute dissection of the duct until it has been fixed in formalin. If the jugular or innominate veins are involved, they are removed along with the

duct; otherwise only the portion of the vein wall containing the entrance of the duct is removed.

In this study no attempt has been made to classify the many minute anatomical variations of the duct. The courses of these ducts fell within the variations listed by Davis[18] in 1915. One duct entered the right subclavian vein at its junction with the jugular. Frequent insulae were present in the thoracic portion, and approximately 15 per cent of the ducts had multiple openings into the jugular, subclavian, or innominate veins. The cisterna was absent or diminutive in about 5 per cent of the cases, and a double duct was encountered in the distal portion three times. Usually there were numerous retroperitoneal nodes present and easily identifiable; the number of nodes draining into the thoracic portion of the duct was less, and occasionally none were identified in this area. No communications with the azygos vein were noted, but injection studies were not performed.

Results and Discussion

In Tables I and II are listed the tumors by type and the incidence of involvement of the thoracic duct and the left supraclavicular lymph nodes. There is an over-all incidence of 40 per cent for ductal involvement and 42.7 per cent for nodal invasion. In the cases of carcinoma the percentage for positive ducts and nodes is 37.2 per cent, while the lymphomas revealed higher figures of 70.6 per cent for ducts and 88 per cent for nodes.

While cases of leukemia and multiple myeloma were not included in the figures given, the ducts and nodes from these cases were studied. In a total of 13 cases of leukemia there were masses of white cells in the ductal lumen of each case, an infiltration of the ductal wall in four instances, and the supraclavicular lymph nodes were involved in 12 cases. In 2 cases of multiple myeloma the duct disclosed no change, but enlarged supraclavicular nodes containing plasma cells were present in both.

Chylous ascites and chylothorax are striking and unusual effusions when fully developed. It is my opinion that more effusions would be classified as chylous if lipid analyses were performed more frequently on those considered doubtful. In this survey definite chylous ascites was present in one case of carcinoma of the esophagus, in 2 cases of bronchogenic carcinoma, and in 3 cases of malignant lymphoma. In each of these there was marked involvement of the duct, particularly where it passed through the diaphragm, a region in which collateral channels are few in number. Lee,[19] by experiments on dogs, found

that he could produce chylous ascites consistently only by ligating the duct where it passed through the diaphragm. Chylothorax occurred in bronchogenic carcinoma once and in malignant lymphoma three times.

TABLE I

Involvement of Thoracic Duct and Left Supraclavicular Lymph Nodes in 129 Cases of Carcinoma (Including Melanoblastoma)

Type of tumor	Total	Ductal involvement	Nodal involvement
Bronchogenic carcinoma	35	18	19
Carcinoma of stomach	14	8	11
Carcinoma of esophagus	14	10	2
Malignant melanoma	10	1	1
Carcinoma of prostate	9	3	6
Carcinoma of colon	10	0	0
Carcinoma of pancreas	6	2	3
Carcinoma of nasopharynx	6	1	2
Carcinoma of liver	3	0	0
Testicular tumors	3	3	2
Carcinoma of kidney	4	0	1
Carcinoma of bladder	3	2	0
Carcinoma of tongue	4	0	0
Carcinoma of tonsil	3	0	1
Carcinoma of skin	2	0	0
Carcinoma of lip	1	0	0
Carcinoma of larynx	1	0	0
Carcinoma of sinus	1	0	0
Total	129	48	48

TABLE II

Involvement of Thoracic Duct and Left Supraclavicular Lymph Nodes in 21 Cases of Sarcoma (Including Neuroblastoma)

Type of tumor	Total	Ductal involvement	Nodal involvement
Hodgkin's disease	5	4	4
Reticulum cell sarcoma	5	3	5
Lymphosarcoma	3	2	3
Unclassified lymphoma	4	3	3
Neuroblastoma	2	0	1
Liposarcoma	1	0	0
Wilms's tumor of kidney	1	0	0
Total	21	12	16

Many cases with ducts showing extensive involvement and dilated tributaries did not show chylous effusion. In one case of lymphatic leukemia chylous ascites was present. Cutting,[20] in 1936, noted this also in leukemia. One of my cases of reticulum cell sarcoma with ductal involvement had white blood cell counts terminally as high as 176,000. These cells were classified as monocytes. This emphasizes the transitions which are known to occur between lymphomas and leukemias.

Numerous single case reports of chylous ascites and chylothorax occur in the literature, but in most the thoracic duct had not been studied. One gains the impression that when neoplasm is the cause, malignant lymphoma is the most common tumor. An excellent review with collection of non-traumatic chylothorax cases is that of Yater[21] who, in 1935, collected 80 cases. Buchanan[22] and Harrell *et al.*[23] in 1939, Jahsman[24] and Olsen and Wilson[25] in 1944 likewise have reviewed cases of chylous effusion in reported series of cases. Consideration of the composition of chylous and pseudo-chylous effusions is given by Wallis and Schölberg[26] and Blankenhorn.[27]

The methods of duct invasion were by lymphatic permeation or embolization from involved local lymph nodes and by direct invasion of the wall. Frequently, the duct was only a channel for transmission of tumor cells, but actual involvement occurred when tumor cells attached to the walls or valves and multiplied. Formation of masses of tumor within the duct often led to obstruction, dilation, and thrombosis (Figs. 5, 9, 11, and 12). Thrombi of the duct may be pure fibrin thrombi or may have many entrapped neoplastic cells. The semilunar valves are very delicate and easily become incompetent. Continuous massive permeation of the duct and its tributaries was observed and had led to thrombosis and tumor involvement of the jugular and innominate veins. The tumorous duct is a source of frequent showers of metastases to the lungs, producing miliary carcinomatosis in some instances. Massive involvement of the duct may lead to prominent dilatation of lacteals in the mesentery and bowel and even to chylous effusion, though the latter is infrequent, because of the extensive collateral channels and anastomoses along the duct.

The manner of involvement of the duct in the lymphomas was almost entirely by direct extension through the wall (Fig. 3). Frequently, masses of neoplastic nodes encircled the duct, producing partial obstruction and marked dilatation of the radicles. There usually was direct infiltration of tumor cells through all layers of the wall. It was obvious, also, that spread can occur from nodes through the

radicles to the duct, since these radicles occasionally contained tumor and the duct was not involved directly.

With carcinoma the spread usually was from the primary tumor to lymph nodes and then through the radicles to the duct where the cells became attached to the wall or formed intraductal masses and cords (Figs. 1, 2, 11, and 12). With anaplastic bronchogenic carcinomas direct extension through the wall in the mediastinal region occurred occasionally. Several bronchogenic carcinomas metastasized to the intra-abdominal viscera, with a secondary spread to the retroperitoneal nodes and the duct from these visceral metastases (Figs. 5 and 11).

Carcinoma of the middle and lower third of the esophagus behaved in a manner different from other tumors, principally because of its proximity to the duct. There was direct extension of the tumor to the duct with subsequent extensive involvement and obstruction, despite the fact that these tumors in general were well differentiated squamous cell carcinomas (Fig. 4). Consistent with the slow growth and spread was the relatively low incidence of supraclavicular nodal involvement. It has been emphasized that this marked obstruction of the duct in carcinoma of the esophagus may play an important rôle in the relatively early debility and weight loss in these cases, for Chêne and Chêne[28] found that there was a significant decrease in blood fat levels in some cases of esophageal carcinoma. Retrograde extension below the point of involvement was seen with regularity only in carcinoma of the esophagus with which implants occurred occasionally on the cisternal walls and valves (Fig. 10). It is possible that retrograde extension into radicles of the ducts occurred in other tumors when ductal obstruction was present, but this was accompanied also by nodal involvement, suggesting that the nodal involvement preceded involvement of radicles and ducts.

In cases of carcinoma the formation of masses of tumor and thrombi in the ducts was relatively common. These lodged at narrow points along the duct, particularly where insulae were present (Figs. 6 and 7). From these points propagation occurred, leading in some instances to a complete filling of the duct. Obstruction of the duct in the cervical portion was observed in a number of cases of carcinoma. High complete or partial obstruction produces a slowing of the flow of chyle allowing formation of thrombi and enhancing the growth of tumor in the duct.

In 5 instances of carcinoma there was thrombosis of the jugular and/or left innominate veins; twice this occurred in bronchogenic

carcinoma (Fig. 5). In each instance there was extensive ductal involvement. The thrombotic material in the veins in 4 of the 5 cases contained tumor. One case of bronchogenic carcinoma, in addition, revealed a small ovoid mass of tumor blocking the upper end of the duct and projecting into the vein (Fig. 8). Scarring and tumor infiltration around the upper end of the duct occurred occasionally and usually resulted from extension of tumor in Virchow's nodes (Fig. 9).

The involvement of the left supraclavicular nodes probably occurred most often by retrograde embolization with increases in intrathoracic pressure and subsequent increase in venous pressure and pressure within the duct itself. Occasionally there was spread to the nodes directly from the primary neoplasm, especially with bronchogenic carcinoma. Attempts were made to palpate supraclavicular nodes at the beginning of the necropsy, and only about one fourth of the nodes showing tumor were palpable. One must remember that these nodes are deeply situated beneath the jugular vein and on top of the scalene muscles. They are surrounded by considerable fatty tissue and covered by fascia. They are probably best approached for surgical excision for biopsy by dissection just lateral to the jugular vein.

These findings certainly suggest that biopsy of these nodes is indicated in many cases of unexplained intra-abdominal and intrathoracic disease just as biopsy of superior mediastinal nodes is used in cases of intrathoracic disease. Such a biopsy may make exploratory laparotomy unnecessary.

Summary

Dissection of the thoracic duct in 150 consecutive cases of tumor death revealed involvement in 37 per cent of the cases of carcinoma and 71 per cent of the cases of lymphoma. The left supraclavicular nodes contained tumor in 37 per cent of the cases of carcinoma and in 88 per cent of the cases of lymphoma. The method of involvement by lymphoma was almost entirely by direct invasion through the wall of the duct. Carcinoma spread to the duct principally through its radicles, but direct invasion was observed with carcinoma of the esophagus and bronchogenic carcinoma. Frequently the involved Virchow's nodes were not palpable. Thus, involvement of the thoracic duct and the left supraclavicular lymph nodes is by no means unusual, and biopsy of these nodes, even if they are not palpable, should be performed more frequently to help establish a diagnosis of intra-abdominal or intrathoracic disease. The thoracic duct is a very frequent and important pathway for the dissemination of neoplastic disease.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Jejunal carcinoma. Of note is the dilated, tumor-filled cisterna. Retroperitoneal and supraclavicular nodes show tumor as well as the upper thoracic duct.

FIG. 2. Malignant teratoma testis. The mass at the lower end of the duct is composed of lymph nodes. The cisterna and lower thoracic portion contain tumor within the lumen. The large mass adjacent to the mid-thoracic segment of the duct is a cystic nodal metastasis.

FIG. 3. Malignant lymphoma. Of note are the numerous neoplastic nodes and the direct growth of the tumor into and around the duct. Virchow's node is attached at the upper end.

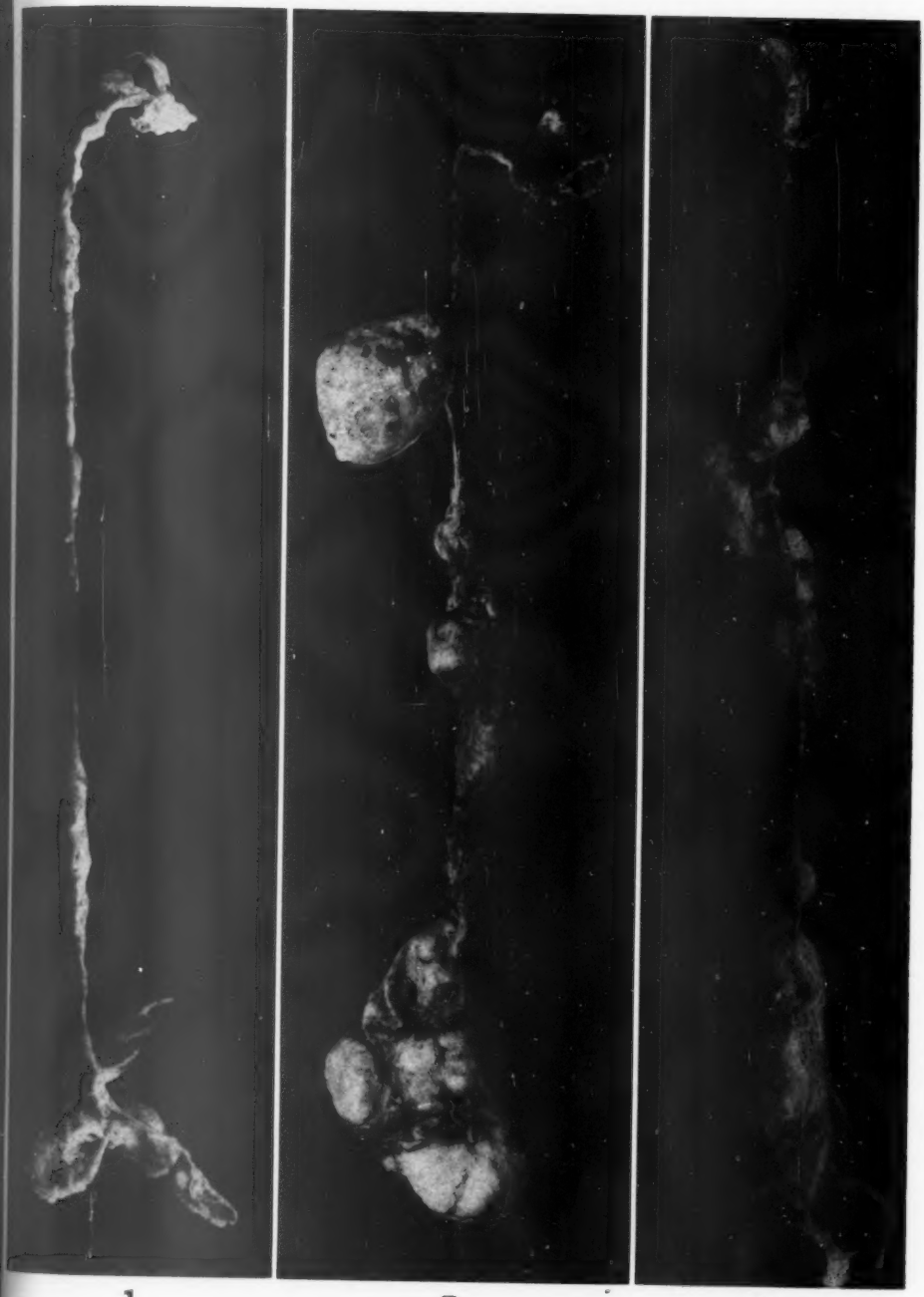

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FIG. 4. Carcinoma of the esophagus. This reveals the usual direct invasion of the duct and adjacent structures. Near the aortic arch the duct is destroyed. There was a mass of tumor implanted in the cisterna.

FIG. 5. Bronchogenic carcinoma. In this instance the duct has become a solid, tumor-filled structure. Tumor has extended into the jugular and innominate veins where thrombosis has occurred.

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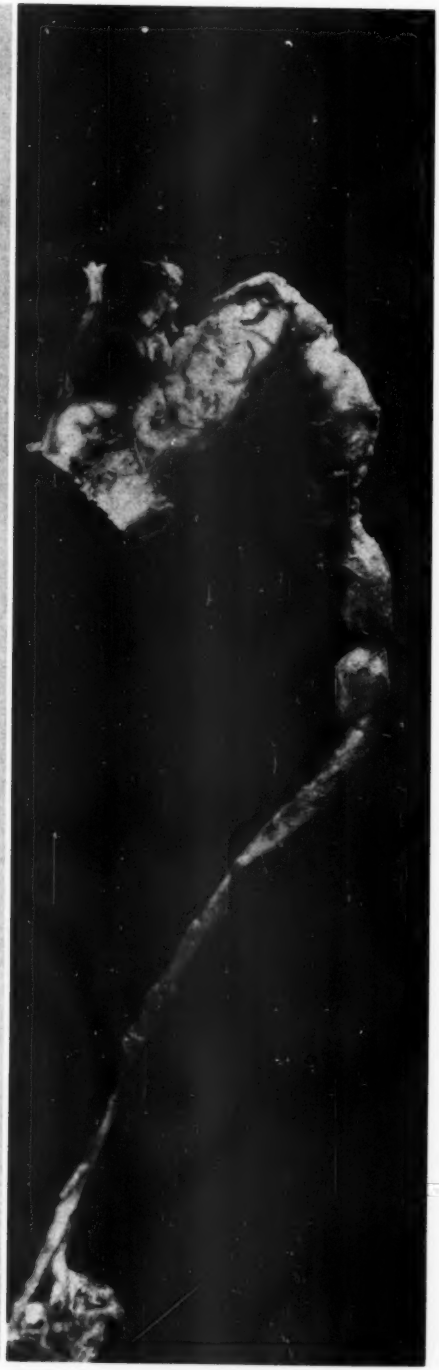

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FIG. 6. Hodgkin's disease. This shows the upper end of the duct and its entrance into the vein (of note are the semilunar valves). The upper division of the duct contains tumor where the insula is formed. There are numerous radicles reaching the duct from the tumorous Virchow's nodes.

FIG. 7. Carcinoma of the stomach. Below the supraclavicular node there is, in the duct, a thrombus which contained tumor. The duct shows three divisions before it enters the vein.

FIG. 8. Bronchogenic carcinoma. Of note particularly is the ovoid mass of tumor blocking the ductal opening and projecting into the vein. Virchow's nodes and a large draining radicle are seen on the right.

FIG. 9. Carcinoma of the pancreas. The duct was a dense cord containing thrombus and tumor with small permeating channels. There is almost complete obstruction where the duct enters the vein.

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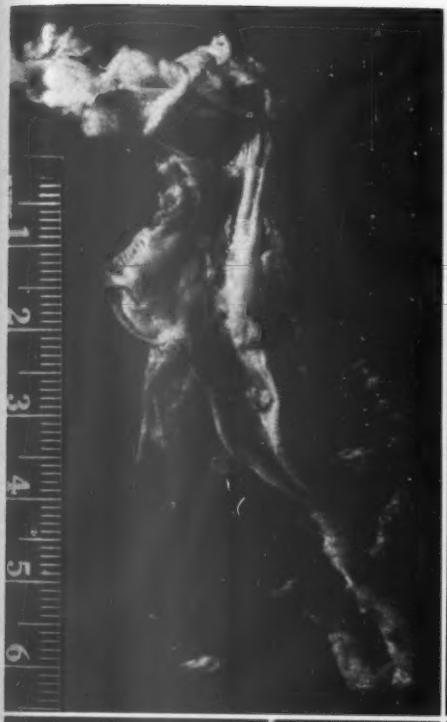
FIG. 10. Carcinoma of the esophagus. This is an opened, dilated cisterna showing numerous granular deposits of implanted squamous cell carcinoma.

FIG. 11. Bronchogenic carcinoma. Of note are the cisterna and its major divisions, all of which are converted to solid tumorous cords.

FIG. 12. Carcinoma of the stomach. Again the cisterna and its branches are filled with tumor. There are numerous anastomosing channels among the branches.

FIG. 13. Malignant melanoma. This was the only instance of involvement with melanoma. There is no cisterna present, but four major branches join to form the thoracic duct. These branches contain clumps of thrombotic material in which there were groups of tumor cells.



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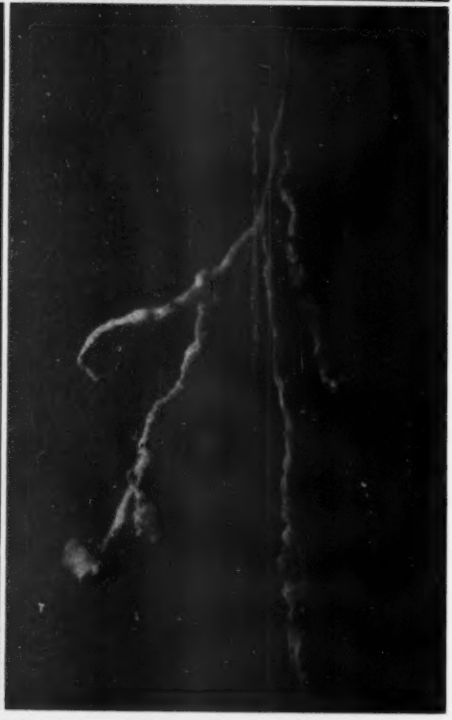

13

MAINTENANCE OF HUMAN NEOPLASM ON THE CHICK CHORIOALLANTOIC MEMBRANE *

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The experiments described in this paper were devised to determine the feasibility of using the chorioallantoic membrane of chicken eggs for the preservation and growth of human neoplastic tissue. Rous and Murphy[1] demonstrated that the chorioallantoic membrane would support the growth of fowl sarcoma and later Murphy[2] was the first to attempt the cultivation of human neoplasms by this method. Only "moderate success" was obtained and no other details of the experiments were mentioned. Goodpasture and his co-workers[3] maintained human skin on the chorioallantoic membrane for 10 days, and by transferring the original graft to other eggs were able to extend the life of the transplanted tissue to 14 days. Human skin was grafted on chorioallantoic membranes by Blank *et al.*[4] Active proliferation of the epidermis and the establishment of chick circulation in the human corium were described. The majority of the original explants survived and some were viable after 4 weekly transfers, i.e., after 27 days. Goodpasture and Anderson[5] also kept grafts of human fetal membrane in a healthy state for 7 to 8 days.

Human cornea was transplanted successfully to chorioallantoic membranes by Kirber and his group[6] who described vascularization of the corneal tissue and proliferation of the corneal epithelium. Rubovits and Abrams[7] grafted human ovarian tissue and endometrium which survived for several days, but serial transfers of the grafts were not attempted. Stevenson[8] attempted to cultivate 8 human neoplasms in eggs, but the results were considered disappointing; after 9 to 12 days no growth had resulted, and the grafts had degenerated. Hurst *et al.*[9] reported experiments with normal and neoplastic human tissues using both duck and chicken eggs. In these experiments malignant and normal cells frequently were able to survive and even to multiply on the chorioallantoic membrane. Ten of 17 tumors and 4 of 5 normal human tissue samples were put on chicken eggs, while duck eggs were used

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for the remainder of the experiments. Thirteen of 17 experiments were successful to some degree. The ability of human tumors to survive more than one transfer has been reported by us.[10] Subsequently, Sommers *et al.*[11] described survival of 28 of 59 different human cancers but did not attempt transplantation beyond the first generation except for one case of epidermoid carcinoma. Dagg *et al.*[12] transplanted over 100 human tumors to the chorioallantois of the chick. The tumors frequently grew or survived during the initial transplant to the chorioallantoic membrane, but failed to grow on serial transplantation. However, this same group,[13] using human tumors that had previously been passed serially in properly conditioned rats and hamsters, were able to maintain one sarcoma for 8 passages, one carcinoma for 6 passages, and one rhabdomyosarcoma for 3 passages.

Methods

All tumors were secured from living patients* and transplanted within 3 hours. The tissue to be inoculated was taken from the midportion of the biopsy specimen and cut into fragments of 1 to 3 mm. The explants were immersed in a solution of penicillin (100 units per cc.) when the chance of bacterial contamination was considered great. The implantation method used was a modification of the one devised by Goodpasture and Buddingh[14] and of the false air sac technique of Burnet.[15] Eggs from white leghorn hens were used for all experiments at 37.2° C. Inoculation of the chorioallantoic membrane was made through a hole, 1 cm. square, in the shell of eggs which had been incubated for 6 to 12 days. The explant was placed near a prominent blood vessel to aid in vascularization and nutrition, and the shell hole was sealed.

When tumor was transferred from one chorioallantoic membrane to another, the membrane around the explant was trimmed carefully and excess membrane removed. The transplant was divided into two to four pieces, representative sections were fixed in 10 per cent formalin and stained with hematoxylin and eosin, and the others were used for further transplantation. These transplants were then placed, membrane side down, on the new membranes.

The details of tissue culture methods used in these experiments were reported elsewhere.[16] Briefly, explants of tissue, 1 to 3 mm. square, were grown using the Maximow double coverslip technique and/or a cellophane coverslip technique. The tissue was maintained 10 to 21 days *in vitro*. Then plasma cultures were cut from the coverslip, avoid-

* We wish to acknowledge with thanks the help of Dr. J. B. Hazard of the Cleveland Clinic in securing some of these tumors.

ing the outer margin of migration, and placed on the chorioallantoic membrane. When perforated cellophane was used, the cellophane disk with the tumor was lifted from the coverslip and inserted tissue side down on the chorioallantoic membrane.

Results

Changes in the Original Explant and the Reaction of the Chorioallantoic Membrane

Eggs were inoculated with 6 malignant human tumors and were sacrificed at periods of from 1 to 11 days. Four thyroid carcinomas, one adenocarcinoma of the breast, and one squamous cell carcinoma of the lung were used. The living tumor tissue on the membrane varied in appearance from white or gray and glistening to dull gray and shriveled. After 24 hours the tumor tissue was adherent to the chorioallantoic membrane (Fig. 1), and the outer cuboidal (ectodermal) epithelium of the membrane in contact with the tumor began to degenerate and the ectodermal epithelium at the margin of the explant, along with the mesenchymal tissue, began to envelop the explant. The chorioallantoic membrane was notably thicker about the margins of the explant. This process continued for 4 to 5 days, at which time the explants were completely encompassed by the mesenchymal tissue between the inner layer and regenerated outer layer of the membrane (Figs. 2 and 3). While these changes were occurring, the base of the explant usually showed signs of blending with the membrane at the place of contact. This occasionally consisted of a pronounced invasion of the base by chick capillaries containing chick nucleated red blood cells. In other specimens the tumor was partially walled off by fibroblasts.

In addition to these changes, there was a reaction of the membrane to the presence of the foreign tissue immediately adjacent to the explant. This was evidenced by the formation of epithelial "pearls" in the mesenchyme, near the explant, and occasionally by extensive proliferation of fibroblasts (Fig. 2) and consequent thickening of the membrane. In several cases there was stratification (Fig. 2) of the outer epithelial layer. These reactions were noted even in sections taken 1 to 2 mm. from the explant. The reaction of the membrane to the explant was more pronounced in eggs in the later stages of incubation (15 to 18 days).

All explants from the 6 tumors survived the incubation period and many cells in each explant appeared healthy (Figs. 1, 2, and 3). In addition, some normal thyroid tissue was included with one of the tumors and it, too, was in a state of good preservation.

Changes Observed During Extended Maintenance of Serial Transplants

Twenty other human tumors were transplanted to chorioallantoic membrane. Fifteen of these tumors survived the primary transplant (Table I).

Seven of the 20 tumors were sarcomas. These sarcomas were implanted upon 137 eggs which lived. The tumors survived on 77 of these eggs (Figs. 4 and 5). Six of the seven strains of sarcoma lived. These six were transplanted to 40 eggs and grew on 13. Three of the six strains survived the second transplant. These, in turn, were transplanted to 10 eggs and one of the three sarcomas remained viable. This tumor survived for a total of 27 days, but after the third graft showed extensive degeneration.

Two astrocytomas and one glioblastoma survived the primary transplant. These were planted on 42 eggs and 24 showed tumor. One astrocytoma survived a second and third transfer. This particular tumor was planted on 6 eggs for the second transfer and all showed tumor. The third transfer was to 2 eggs which survived and both showed tumor. Thus, one astrocytoma survived 3 transfers for a total of 22 days (Figs. 6 to 9).

TABLE I

Survival of Serial Transplants of Human Neoplasms on the Chick Chorioallantoic Membrane

Type of tumor	No. of specimens	1st Transplant	2nd Transplant	3rd Transplant
Sarcoma	7	6	3	1
Glioma	3	3	1	1
Breast carcinoma	4	3	0	0
Thyroid carcinoma	2*	1	0	0
Melanocarcinoma	2	1	0	0
Neurilemoma	1	1	1	0
Renal cell carcinoma	1	0	0	0
Totals	20	15	5	2

* For one of the thyroid carcinomas, the surviving material recovered from the eggs was not neoplastic tumor, but well developed, normal, adult thyroid tissue. This was not counted as a successful transplant in this table.

Three of the four breast carcinomas survived the first transfer, although all subsequent transplanting failed. Twenty-three of 70 eggs which were planted and which lived contained tumor. However, for one of the tumors, 15 eggs were planted and all 15 showed tumor at the end of a 5-day period.

One thyroid carcinoma survived the initial transplant. Of 26 eggs which were planted, 15 showed tumor but none of the explants survived the second transfer.

One neurilemoma was planted on 4 eggs that lived. Two eggs showed tumor. These two fragments were cut up and regrafted on 4 eggs, 2 of which again showed tumor. This represented a survival of 15 days (Figs. 10 and 11).

One of two melanocarcinomas survived the first graft (Figs. 12 and 13). This one was planted on 12 eggs, 6 of which contained tumor but none survived the second graft. The other was planted on 25 eggs and none of the fragments survived.

One renal cell carcinoma was planted on 4 eggs but no positive takes were obtained.

Tissue Culture Implants on Chorioallantoic Membrane

Explants from tissue cultures of tumors were transplanted to eggs. The results are tabulated in Table II. The tissue cultures used for these experiments were judged as acceptable for transplanting on the

TABLE II

Survival of 11 Human Tumors in Chorioallantoic Membrane after Maintenance in Tissue Culture

Tumor	Time in tissue culture	Time in chorioallantoic membrane	Number of eggs	Number of successful transplants
	(*days*)	(*days*)		
Carcinoma of urinary bladder	16	8	6	1
Carcinoma of breast	16	8	4	0
Carcinoma of breast	21	8	4	1
Carcinoma of thyroid gland	16	8	6	0
Carcinoma of thyroid gland	10	10	5	1
Carcinoma of thyroid gland	19	9	6	1
Carcinoma of thyroid gland	18	7	12	6
Carcinoma of thyroid gland	8	11	3	0
Hemangiopericytoma	19	10	4	0
Giant cell tumor of bone	21	3	13	2
Squamous cell carcinoma	13	4	3	2
Totals			66	14

basis of microscopic appearance, and on pH changes in the media which indicated that metabolism was taking place. The main difficulty encountered in using this technique was that the entire implant disappeared in many cases and therefore a large number of implants are not included in the results. The transplants were successful in 7 of 11

different tumors that were first maintained *in vitro* for 10 to 21 days and then placed in the chorioallantoic membrane for 3 to 11 days. More precisely, of a total of 66 tissue culture transplantations to eggs, only 14 survived on the chorioallantoic membrane for a period of 3 to 11 days. The ability to survive on the chorioallantoic membrane after previous growth or maintenance *in vitro* was not enhanced. The preservation of tissue by the combined technique was more troublesome and involved and did not produce as good results.

Discussion

When human tumor tissues were maintained on the chorioallantoic membrane of the chick embryo during one incubation period, the results were essentially successful and the original histologic characteristics, generally, were well preserved. Less striking results were obtained when the tumors were transferred serially. It is of interest that the best survivals, in the more extended experiments, have been with tumors of connective tissue origin and with the gliomas.

The question of whether actual multiplication of cells occurred has received special attention by others. Stevenson[8] reported that no mitotic figures were seen in his implants of human tumors, although they were seen in the animal tumors. However, Hurst *et al.*[9] claim to have observed mitotic figures and so mention actual growth on a membrane. In our material mitotic figures were observed infrequently.

Epithelial pearls, fibroblastic proliferation, keratinization, and stratification of membrane epithelium were all observed to be a reaction of a part of the membrane to the presence of a foreign tissue. These reactions were not constant, but on the other hand their absence could not be correlated with any particular type of tissue. The usual picture involved a slight stratification of epithelium, one or two pearls, and a collection of fibroblasts around the tissue, to form a ring. This type of membrane response has been described in more detail for other tissue explants by Campbell.[17]

The question of the feasibility of this technique for extended preservation of human tumor arises. If it is to have practical application, some adjuvant must be found which would result in a longer period of viability. It was not possible to preserve tissue in good condition by these methods for longer than 10 to 15 days with any assurance of positive results. Indeed, 1 week would appear as a more secure time limit to set. In our material, of the 15 tumors which survived and in which serial transplants were attempted, only 5 survived two graftings and only 2 survived three graftings.

The reason for a positive take in some instances and a negative

result in others is not evident. It is possible that different original cell populations alone could account for this or the explanation may lie with peculiarities of the membrane-tissue base relationship or even with trauma incident to the procedure. In discussing this problem Hurst *et al.*[9] remarked that tissue often disappears from inoculated membrane, a difficulty which we experienced also, especially with small bits of tissue from tissue culture. However, this did not occur so often with the larger implants, which is somewhat surprising, since smaller implants obtain nutriment more easily.

Previous adaptation of tumor tissue *in vitro* did not enhance the subsequent survival on the chorioallantoic membrane.

Summary

The survival of human tumor tissue on the chorioallantoic membrane of developing chick eggs was investigated with 26 different tumor samples. Six tumors were studied during the time of one incubation period only. In addition, 20 tumors were transplanted with the intention of prolonging survival by serial transplants. Five of these did not survive the initial transplanting. Of the 15 that survived the initial transplant, 5 survived the second transplant. Of these 5, 2 survived an additional third transplant. In additional experiments, 11 tumors, which were first cultivated *in vitro*, were transplanted onto the chorioallantoic membrane. This more complicated procedure did not enhance the results.

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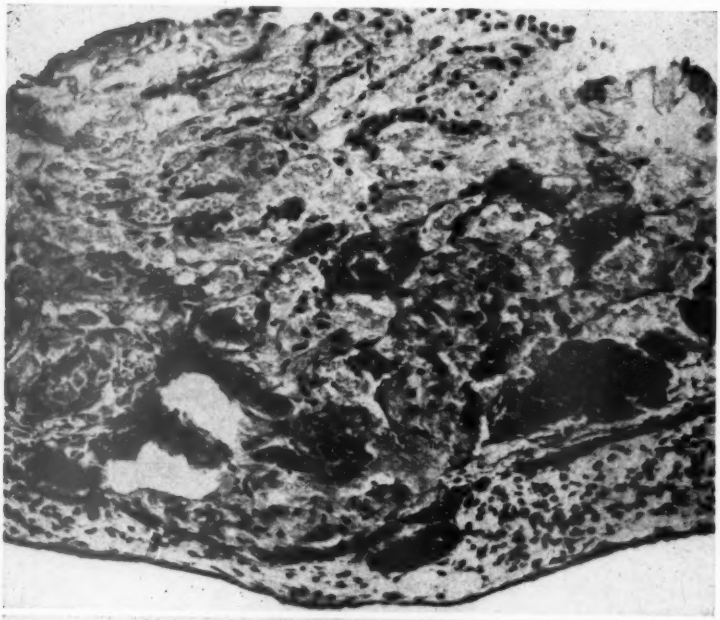
LEGENDS FOR FIGURES

FIG. 1. Thyroid carcinoma: 1-day-old primary transplant. Chorioallantoic membrane below. Many of the tumor cells have survived although some have degenerated. Hematoxylin and eosin stain. × 185.

FIG. 2. Thyroid carcinoma: 4-day-old primary transplant. The tumor is completely surrounded by an epithelium-lined layer of vascular chorioallantoic membrane and chick fibroblasts. At the base is a dense zone of chick fibroblasts, capillaries and vascular "pools" containing chick erythrocytes. There is stratification of the chorioallantoic membrane epithelium at the margins of the illustration. Hematoxylin and eosin stain. × 100.



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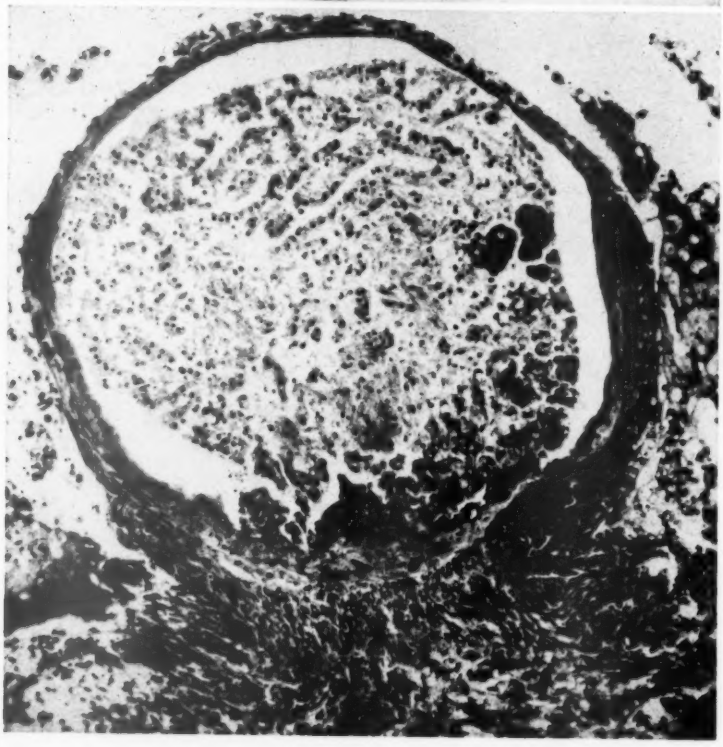

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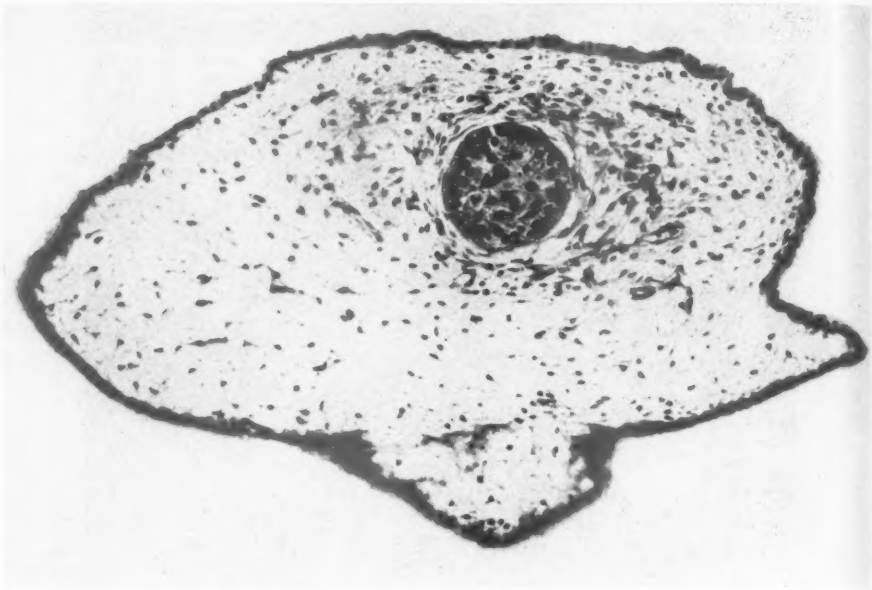
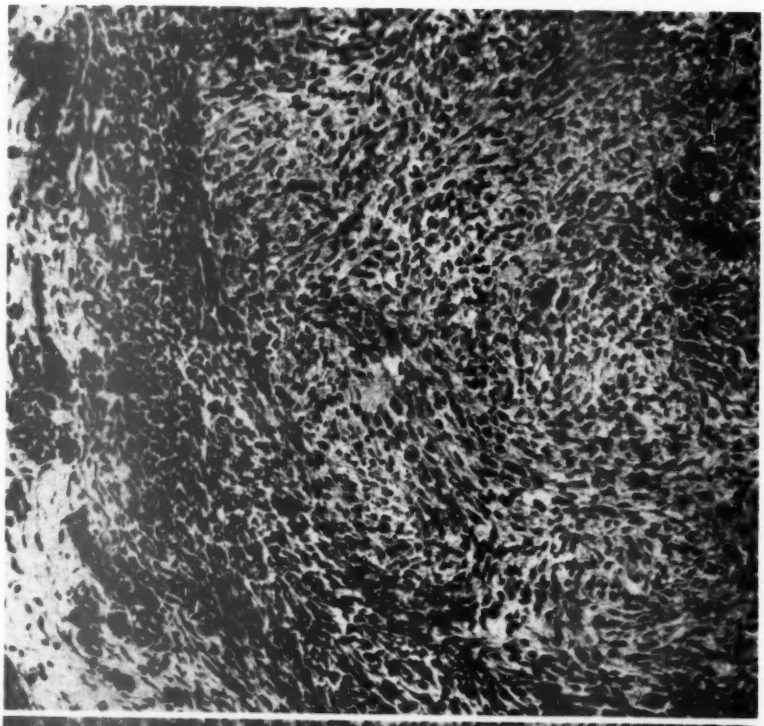


FIG. 3. Squamous cell carcinoma: 4-day-old primary transplant. The tumor is completely enveloped by chorioallantoic membrane and surrounded by fibroblasts. Of note are the atypy and variability of the cells and a few mitotic figures. Hematoxylin and eosin stain. × 185.

FIGS. 4 and 5. Fibrosarcoma. *Fig. 4.* Eight-day-old primary transplant completely incorporated into the chorioallantoic membrane which is shown in the lower left corner. Some mitotic figures are present in the tumor. Hematoxylin and eosin stain. × 185. *Fig. 5.* Photomicrograph of original tumor for comparison with Figure 4. Hematoxylin and eosin stain. × 185.



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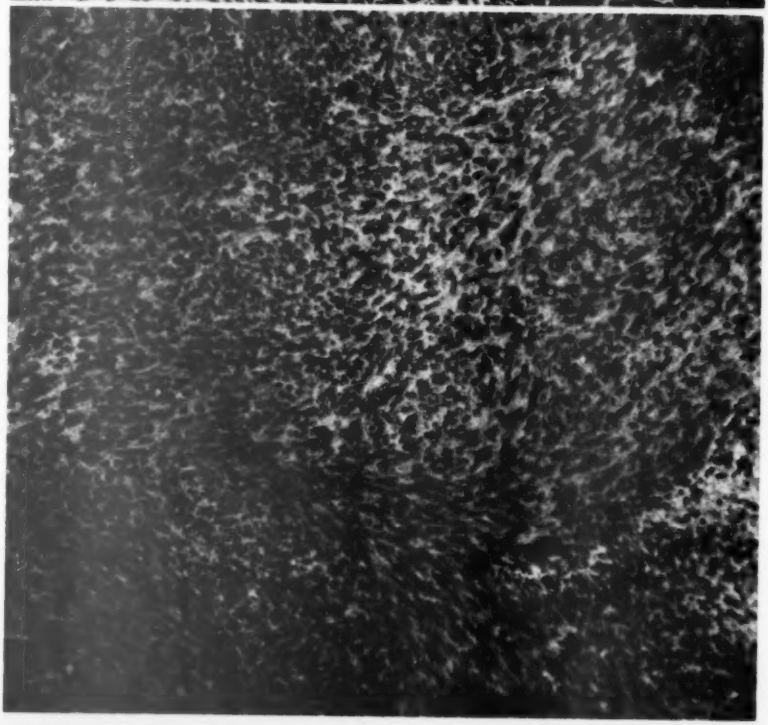

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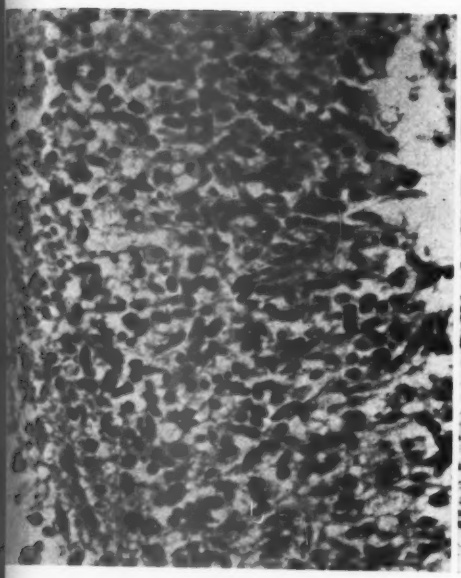
FIGS. 6 to 9. Astrocytoma. *Fig. 6.* Seven-day-old primary transplant. Nucleated chick erythrocytes are present in the transplant. Hematoxylin and eosin stain. × 330. *Fig. 7.* Second transplant, 15 days. Hematoxylin and eosin stain. × 330. *Fig. 8.* Third transplant, 22 days. Viable tumor tissue remains even though there is some replacement by chick fibroblasts. Hematoxylin and eosin stain. × 330. *Fig. 9.* Photomicrograph of original tumor. Hematoxylin and eosin stain. × 330.



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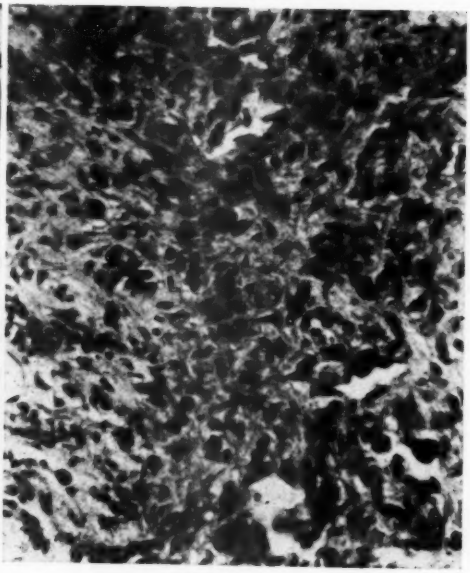

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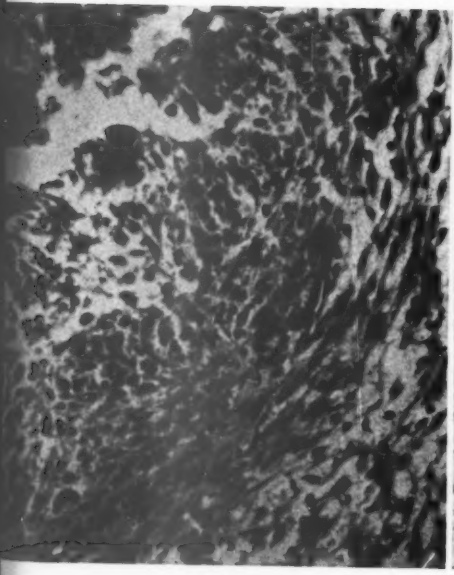

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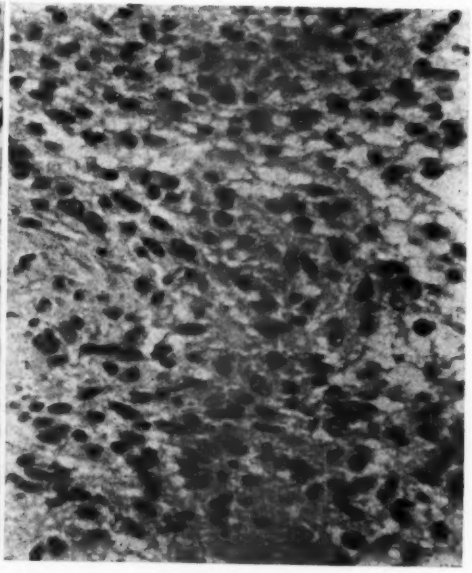

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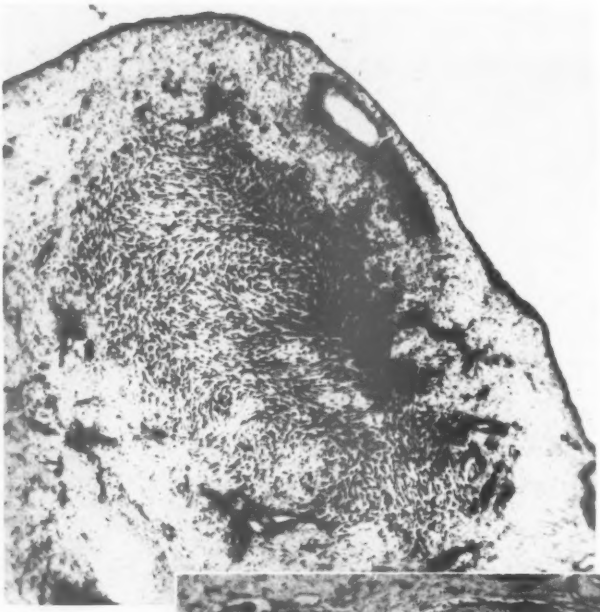

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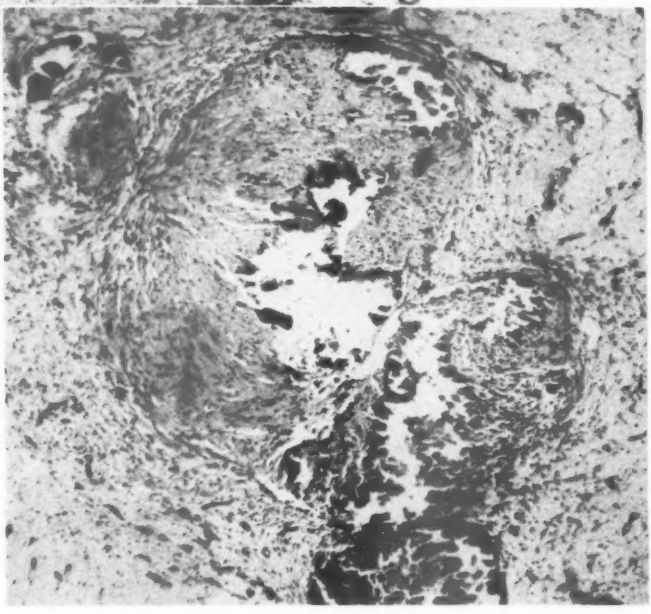
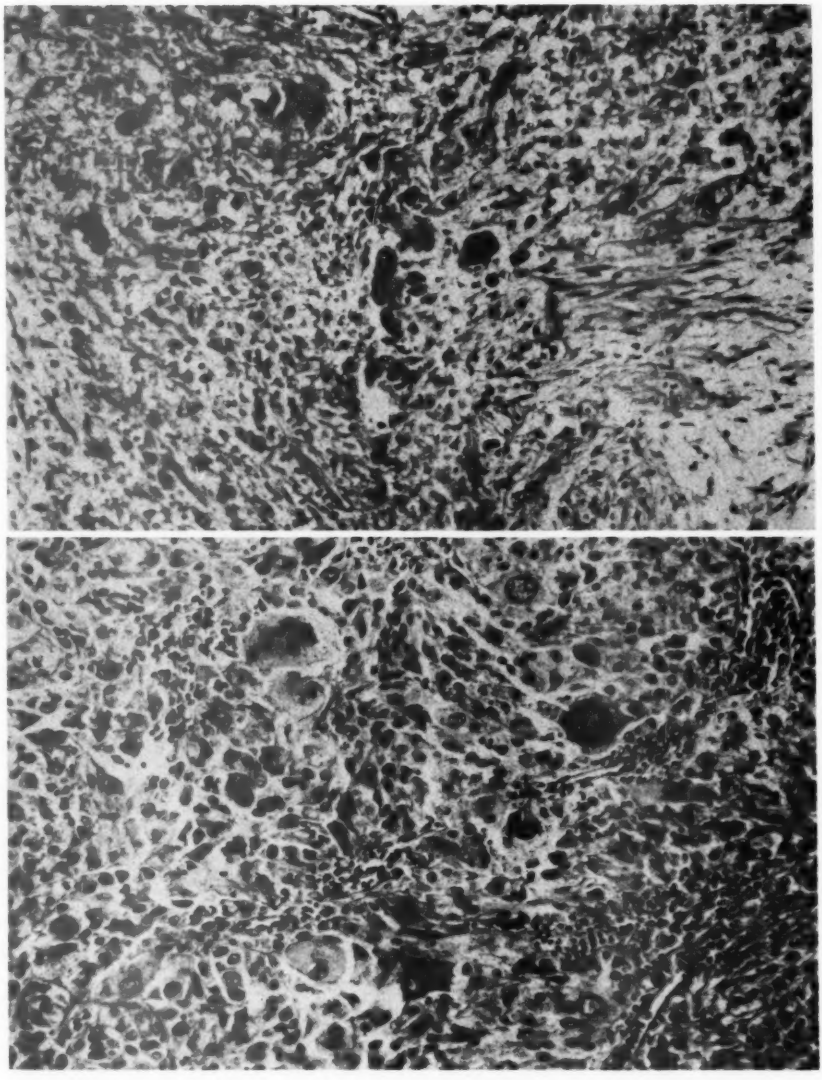


FIGS. 10 and 11. Neurilemoma. *Fig. 10.* Eight-day-old primary transplant. Tumor enveloped by chorioallantoic membrane. Whorls of cells are seen. Hematoxylin and eosin stain. × 75. *Fig. 11.* Second transplant, 15 days. There is an area of necrosis in the central portion of the well preserved tumor. Hematoxylin and eosin stain. × 60.



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FIGS. 12 and 13. Melanocarcinoma. *Fig. 12.* Seven-day-old primary transplant. There is necrosis of tumor cells, but well preserved groups of cells are scattered through the field. Hematoxylin and eosin stain. × 185. *Fig. 13.* Photomicrograph of original tumor for comparison with Figure 12. Hematoxylin and eosin stain. × 185.

PULMONARY MUCORMYCOSIS *

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Pulmonary mucormycosis appears to be a new disease in the United States, and one of increasing frequency. The first case in this country was published in 1948, by Baker and Severance,[1] while 5 cases have been recognized in the past 1½ years in North Carolina and South Carolina alone. Had this disease been prevalent in this country earlier, it would have been recognized and reported, since it is readily recognized in hematoxylin and eosin sections and presents distinctive arterial lesions.

The increased incidence of mucormycosis, like the increased incidence of candidiasis, is probably due to the increasing use of drugs which inhibit bacterial infections. This situation permits intercurrent and terminal mycotic infection to develop in cases which previously would have displayed bacterial pneumonias. The increasing use of chemotherapy in leukemia and malignant disease and the use of cortisone and ACTH are other factors which may favor mycotic infection when bacteria are suppressed.

Recent reports of mucormycosis have emphasized infections of the central nervous system. For example, Gregory, Golden, and Haymaker,[2] in 1943, described 3 cases in persons who died in diabetic coma with meningo-encephalitis, thrombosis of the internal carotid artery or other vessels, and intra-orbital cellulitis. Several similar fatal cases have been observed.[3-5] An orbital case, with recovery, has been followed in the Department of Pediatrics of Duke University School of Medicine.[6]

Since the pulmonary case of Baker and Severance[1] was recorded only in abstract form, a full account will be given later in this paper, as case 4 (Table I).

A second case was reported in 1949 by Lloyd, Sexton, and Hertig[7] of Boston in a parturient woman with clinical features suggesting diabetes. The lesion was localized in the right upper lobe. A third case was reported in 1954 by Martin *et al.*[8] in an infant who died of the intra-orbital and meningeal form of the disease with bilateral thrombosis of the internal carotid arteries. Pulmonary lesions were part of

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TABLE I

Recent Cases of Pulmonary Mucormycosis

Case no.	Author	Age	Race, sex	Residence	Predisposing factor	Duration of mycosis	Pulmonary lesions	Cause of death
						days		
1		47	White M	N.C.	Leukemia, cortisone, ACTH, antibiotics	30	Thrombosis, massive infarct, pneumonia, pleurisy, right lung; mycotic bronchitis	Pulmonary mucormycosis
2		23	White M	N.C.	Leukemia, 6-mercaptopurine, antibiotics	19	Thrombosis, infarct, right lower lobe; mycotic bronchitis	Pulmonary mucormycosis
3		51	White M	S.C.	Diabetes	13	Thrombo-arteritis, thrombophlebitis, mycotic pneumonia, left hilus; mycotic bronchitis	Pulmonary mucormycosis
4	Baker and Severance[1]	3	White F	Texas	Diabetes	10	Thrombo-arteritis, mycotic pneumonia, mycotic bronchitis	Orbital, cerebral, and pulmonary mucormycosis
5		50	Negro M	N.C.	Diabetes	8	Thrombo-arteritis, mycotic pneumonia, right upper lobe	Diabetic coma, renal insufficiency, pneumonia, mucormycosis incidental
6		3	Negro F	N.C.	Burns, penicillin	3	Thrombo-arteritis, left upper lobe	Burns and vascular mycosis
7		3	White M	Ariz.	None known	30	Thrombosis, infarcts	Disseminated mucormycosis, pulmonary lesions as contributory factor
8	Lloyd, Sexton, and Hertig[7]	26	Negro F	Mass.	Pregnancy, diabetes(?)	13	Thrombophlebitis, infarct, right upper lobe	Pulmonary mucormycosis
9	Martin *et al.*[3]	2½	White M	Texas	Diarrhea, chemotherapy	8	Hematogenous foci, fibrinous pleurisy	Orbital and cerebral mucormycosis, pulmonary mucormycosis incidental
10	Zimmerman[8]	53	Negro M		Multiple myeloma, urethane, cortisone, roentgen therapy		Infarct	Cardiac infarct

the disseminated disease demonstrated at necropsy. Zimmerman[8] has referred briefly to a fourth case with pulmonary lesion, but with death due to mucormycosis of the heart.

Six additional cases of mucormycosis are now reported. They are cases numbered 1 to 3 and 5 to 7 in Table I.

Report of Cases

Case 1. Mucormycosis of Right Lung with Massive Infarct in Leukemic Patient

V. A., a 47-year-old white male, native of North Carolina, developed symptoms of acute myeloblastic leukemia 11 months before death. Five months after the onset of symptoms he was found to have an enlarged liver and spleen. Examination of the blood showed hemoglobin, 6.5 gm.; white blood cells, 1,800 per cmm. with a differential count of polymorphonuclear leukocytes, 21 per cent; staff cells, 1 per cent; myeloblasts, 8 per cent; small lymphocytes, 28 per cent; large lymphocytes, 38 per cent; monocytes, 4 per cent. In the bone marrow, 95 per cent of the cells were myeloblasts. The patient was treated with cortisone acetate, 200 mg. per day, and procaine penicillin, 600,000 units per day. The cortisone was continued.

A month before death the patient developed severe right anterior chest pain and a cough productive of bloody sputum. Two weeks before death he entered the hospital. A roentgenogram showed a homogeneous density of the central one third of the right lung space (Fig. 1). There was a pleural friction rub on the right. The blood now showed hemoglobin, 9.5 gm.; hematocrit reading, 24; white blood cells, 2,900 per cmm. with a differential count of polymorphonuclear leukocytes, 66 per cent; staff cells, 13 per cent; small lymphocytes, 17 per cent; large lymphocytes, 4 per cent. Cultures of sputum were negative for fungi and acid-fast organisms. He was treated with penicillin and streptomycin. The cortisone acetate was tapered off and ACTH gel, 40 units every 6 hours, was started.

Several thoracenteses failed to produce fluid. He died with gasping respirations and cyanosis.

Gross Findings

The right pleural cavity was obliterated by fibrous adhesions except for locules which contained a total of 200 cc. of thin, pink fluid. The right lung weighed 2,000 gm., was bulky, and failed to collapse. It was covered with thick, edematous, and hemorrhagic adhesions and fibrinous exudate, and the lobes were densely bound together. Most of the lung was hard; only the apex and base were soft. The right leaf of the diaphragm was thick, edematous, and hemorrhagic, with fibrin covering both upper and lower surfaces. On section, it was evident that the hard mass was a massive infarct which involved all lobes except for an emphysematous, apical region and an air-containing zone close to the diaphragm (Fig. 2). The right pulmonary artery and several of its branches were filled with grayish red thrombi. The right pulmonary vein was unobstructed but tributary veins contained thrombi. The right bronchial tree contained soft gray material. The left lung was emphysematous and air-bearing.

The other organs of the body presented the features of leukemia. The enlarged spleen weighed 420 gm., while the lymph nodes of the mediastinum and axillae were larger than normal, soft, and gray. The brain was not removed.

Material taken for culture from the anterior aspect of the right lung failed to grow fungi.

Microscopic Findings

On microscopic examination, masses of broad, branching hyphae lay in the lumina of large bronchi and penetrated their walls. Similar hyphae were numerous in the fibrous and fatty hilar tissues, and in lymph nodes. They permeated the walls and lumina of the right pulmonary artery and its branches, and produced thrombi (Figs. 3 and 4). No organization was seen in the thrombi. Walls of veins were similarly involved, with thrombi within them also. In the infarct of the lungs many shrunken hyphae were seen in the necrotic and hyaline tissues.

Section through viable pulmonary tissue showed hyphae in alveoli with edema fluid, neutrophils, fibrin, and red cells. There was fibrinous pleurisy with regions of fibroblastic organization. Appropriate stains failed to demonstrate bacteria in the lung.

The swollen diaphragm showed acute, necrotizing, edematous and hemorrhagic myositis with many hyphae (Figs. 5 and 6).

Leukemic infiltrates were noted in spleen, lymph nodes, and marrow. Vertebral and femoral bone marrow consisted largely of blast cells.

Comment. Pulmonary mucormycosis was the immediate cause of death.

Case 2. Mucormycosis of Lung with Infarcts in Leukemic Patient

J. M., a 23-year-old white male, native of North Carolina, developed granulocytic leukemia 18 months before death, with white blood cell counts as high as 700,000. There were cervical and axillary lymphadenopathy, hepatosplenomegaly, and subcutaneous ecchymoses. Six months before death the patient had pneumonia of the left lower lobe, with pleural fluid. Three weeks before death stomatitis and ulceration of the tongue developed and were complicated by bacterial and monilial infection. Nineteen days before death pneumonia developed on the right side. Treatment of the leukemia had consisted of 6-mercaptopurine, while antibiotic drugs were used to combat infection. Cortisone was given during the last few days of life.

The final clinical impressions were: granulocytic leukemia; nasopharyngitis; extensive bronchopneumonia, involving all lobes, with lobar consolidation at the right base; fibrinous pleurisy, right; agonal ulceration of gastro-intestinal tract with hemorrhage; ulceration of tongue; ecchymoses and petechiae of skin.

Gross Findings

The right pleural cavity contained 2,500 cc. of hemorrhagic fluid. The right lung weighed 800 gm. The pleural surfaces presented fibrous adhesions and fibrinous exudate. The right lower lobe felt firm. On section, the upper lobe presented a small, hemorrhagic, firm region in the anterior portion. The middle lobe had a similar region medially. The lower lobe was red, lumpy, and consolidated, with an abscess 1 cm. in diameter in the anterior portion. All lobes on the right were congested and edematous. There were clots in intrapulmonic arteries. The peribronchial nodes were enlarged, and gray on cut surfaces. The bronchi contained pinkish white mucus.

The left pleural cavity contained 1,000 cc. of hemorrhagic fluid. The left lung weighed 650 gm. In the upper lobe there were two infarcts, each 3 cm. in diameter. The lower lobe was congested. The lymph nodes were enlarged; there were clots in arteries; and pinkish white mucus occurred in bronchi.

The liver weighed 2,550 gm. and the spleen, 1,150 gm. There were ecchymoses of skin and mucous membranes; ulcers of esophagus, stomach, and intestine; hemorrhage in the stomach (200 cc. of coffee-ground material); and hemorrhagic fluid in the intestines. Peyer's patches and lymph nodes were swollen.

Microscopic Findings

Microscopically, there was organizing fibrinous pleurisy over the right upper lobe, and hyaline thrombi in pulmonary capillaries. The right middle lobe was covered with fibrinous exudate and there was edema fluid in the alveoli. Small blood vessels were filled with hyphae. The right lower lobe was occupied largely by an infarct with thrombosed vessels and peripheral organization. Coenocytic, branching hyphae with rare septa measured 6 μ in thickness, except for occasional bulgings which were 12 μ in thickness. Bronchi contained similar hyphae. The "abscess" was a softened region of the infarct with masses of cocci but without neutrophils. The left lung presented organizing fibroblastic plugs in alveoli, without hyphae.

The spleen contained an infarct in which hyphae occurred. Leukemic infiltrates were demonstrated in spleen, lymph nodes, testes, renal capsule, and elsewhere.

Comment. Death was due to pulmonary infarction and pneumonia, with the fungus of mucormycosis the predominant infectious agent, but with cocci present. The cause of the organizing pneumonia of the left lung was not established.

Case 3. Mucormycosis of Hilus of Left Lung in a Diabetic Patient

E. H. was a white male diabetic patient, 51 years old, who had had a gangrenous toe amputated 3 years before death. The following year he was hospitalized for 2 weeks because of a prostatic abscess. His diabetes had been controlled by diet although he had taken insulin occasionally. He had hypertension and mild anemia.

Thirteen days before death he developed fever, vomiting, and mild diarrhea, with shaking chills each night. Three days before death he developed cough and dyspnea, and râles were heard on the left. A roentgenogram revealed a shadow at the left hilus which involved one third of the lung field. In another roentgenogram made the day before death, there was increase in the size of the infiltration of the left lung (Fig. 7). Laboratory studies 3 days before death showed hemoglobin, 9.6 gm.; white blood cells, 23,550 per cmm., with a differential count of neutrophils, 94 per cent; staff cells, 3 per cent; lymphocytes, 3 per cent. The urine showed 3 plus albumin, 3 plus sugar, and 4 to 6 white blood cells per microscopic field. Chemical examination of the blood showed icterus index, 4.6 units; CO_2-combining power, 30 volumes per cent; blood sugar, 400 mg. per cent; non-protein nitrogen, 65 mg. per cent. The blood sugar the day before death was 340 mg. per cent and the CO_2 capacity, 44 volumes per cent. Therapy consisted of parenteral fluids, insulin, penicillin, streptomycin, and erythromycin. The patient continued to be febrile with a spiking fever to 103° F.

Gross Findings

The hilus of the left lung, over an area 12 by 15 cm., was firm and dark reddish blue, with edema and congestion. The left pulmonary artery and its branches were filled with putty-like, soft, yellow-gray material that could be expressed with light pressure. The bronchi contained similar material. The left lung weighed 750 gm.; the right, 400 gm.

The spleen weighed 250 gm. Other organs of the trunk were not remarkable.

Microscopic Findings

Microscopically, the bronchi of the left lung contained masses of broad hyphae and numerous gram-positive cocci (Fig. 8). The lining epithelium was often absent and hyphae could be seen within the walls of the bronchi. The fatty and fibrous tissue about the hilar vessels was infiltrated with hyphae and neutrophils. Lymphatics contained fibrin, neutrophils, thrombi, and hyphae. Veins and arteries of various sizes in the hilar region showed hyphae in the walls and some dense collections of neutrophils and flakes of hyalinized fibrin in the lumina. There was patchy pneumonia associated with hyphae. It was most frequently of a purulent type, but at times fibrinous or edematous. Hyphae averaged 12 μ in width. No cocci were stainable in the pneumonia.

Other observations included chronic pyelonephritis, glomerulosclerosis, acute splenic tumor, generalized arteriolosclerosis, coronary atherosclerosis, and cardiac hypertrophy.

Comment. Death was caused by mucormycosis, since the terminal features were those of infection. There was insufficient acidosis to indicate a diabetic death, and the evidences of kidney disease were insufficient to indicate a renal death.

Case 4. Mucormycosis of Lungs, Orbit, Meninges, and Cerebrum, with Multiple Thrombi

This is the case of Baker and Severance,[1] reported previously in abstract.

A white female child, 3 years old, died on March 11, 1947, in San Antonio, Texas. She had had symptoms of diabetes for 1 year, including polyphagia, polyuria, polydipsia, and severe weight loss; and insulin had been used in treatment. The urine contained sugar (1 plus); a glucose tolerance curve was diabetic in type. Ten days before death she began to cough, became febrile, and vomited. Five days before death she was semicomatose, with grunting respiration and bilateral moist râles, more marked on the left posteriorly. The eyeballs were soft. Examination of the blood showed hemoglobin, 78 per cent or 11 gm.; red blood cells, 4.2 millions; white blood cells, 15,600 per cmm. with a differential count of staff cells, 16 per cent; segmented cells, 63 per cent; lymphocytes, 20 per cent; monocytes, 1 per cent. A urine specimen never could be obtained. Severe convulsive seizures, cyanosis, and coldness developed on the night of admission. Two days before death the child vomited "coffee-ground" material, and this continued to drain from the nose and mouth. The day before death the left pupil was much larger than the right, and there was hemorrhage in the left eye.

Gross Findings

The lower lobe of the left lung was congested and edematous on cut section, and there was a dark purple area, 4 cm. in diameter, in the middle of the lobe with thrombi in branches of the pulmonary artery. The bronchi contained thick, brown, mucinoid material. The upper lobe on the left was pink and air-containing. The upper right lobe was congested and edematous on cut section, with a severely congested area 2 cm. in diameter. There was a thrombosed vein leading from this lobe. The middle lobe was dark purple, but air-containing. The lower lobe was pink, edematous, and rubbery. The bronchi on the right contained more of the thick, brown, mucinoid material noted on the left.

In the spleen there were wedge-shaped infarcts, the largest 7 mm. in diameter, and yellow flecks on the cut surface. The other organs of the trunk appeared normal.

On the inferior surface of the left frontal lobe of the brain there was an area of injection and softening which measured 4 by 2.5 cm.; and there were similar areas over the tip of the left temporal lobe, 3 by 2.5 cm., and on the inferior surface of the right frontal lobe, 2.5 by 1 cm. Section through these areas showed a hemorrhagic and soft cortex. The left internal carotid artery presented a thrombus at the level where it was severed in removing the brain. There also were

thrombi in vessels of the left middle fossa. In contrast to the ordinary yellow fat of the right orbit, the left orbital fat was congested and red.

Microscopic Findings

Microscopically, sections of bronchi of all sizes contained branching hyphae, 4 to 18 μ thick and up to 100 μ long, with occasional neutrophils and red cells. The wall of the largest bronchus was necrotic. The pulmonary parenchyma was not necrotic and contained numerous hyphae like those in the bronchi (Fig. 9). Alveolar exudate consisted of edema fluid, hemorrhage, fibrin, and small numbers of neutrophils. The larger hilar blood vessels presented hyphae everywhere, including, especially, permeation of walls (Fig. 10). Thrombi, estimated to be of several days' duration, also showed hyphae. They occluded all but an axial pathway for fluid blood. Organization of thrombi was not observed. Many of the smaller vessels contained fluid blood. Lymph vessels in edematous pulmonary fibrous septa contained hyphae and neutrophils (Fig. 11). Since all of the numerous sections of lung showed hyphae, it was presumed that the infection involved all lobes of both lungs.

There were fungi in hilar lymph nodes, fat, connective tissue, perineurial lymphatics, nerve trunks, and pleura.

The spleen contained small, recent infarcts without fungi, and clusters of lipophages. These fatty macrophages were found in the liver also. Radial foci of fibrosis and lymphocytic infiltration were seen in the kidneys.

Branching hyphae were numerous in the thrombosed left internal carotid artery and in orbital tissues, including arteries, veins, perineurial lymphatics, edematous muscles, and bone. Arteries and veins were thrombosed. There were hyphae in and about thrombosed meningeal and intracerebral vessels, free in the brain substance, and in the capsule of the pituitary body. The leptomeninges in some segments were normal; in others, infiltrated with neutrophils and round cells. There was necrosis of brain substance, with minimal acute inflammation. When free in the brain tissue, the fungus often had no surrounding inflammatory reaction. The cerebellum, pons, cord, and medulla appeared normal.

There were gram-positive rods and yeast cells in a large bronchus; but no bacteria in pulmonary parenchyma, orbit, or brain. Cultures of the fungus were not obtained.

Comment. The mycotic pneumonia of this case probably was secondary to the orbital infection. Other diagnoses included chronic healed pyelonephritis and lipid histiocytosis of spleen and liver.

Case 5. Terminal Mucormycosis of Right Lung, Mixed Fungus Flora of Left Lung with Cavity, Diabetes Mellitus with Chronic Pyelonephritis, and Terminal Acidosis and Uremia

H. C., a 50-year-old colored male, came to Duke Hospital 2 years before death for the control of diabetes which he had had for 6 years. He had lost 70 pounds in weight. The diagnoses were diabetes and neuropathy, syphilis of the central nervous system, and non-toxic goiter. He was discharged with the diabetes under control by diet, without insulin.

Five days before his terminal admission he became delirious. On admission, 3 days before death, he was comatose, dehydrated, emaciated, but afebrile. Examination of the blood showed white blood cells, 28,900 per cmm. with a differential count of polymorphonuclear leukocytes, 79 per cent; staff cells, 14 per cent; small lymphocytes, 4 per cent; monocytes, 3 per cent. The urine showed pH, 7.0; protein, 3 plus; sugar and acetone, negative. Chemical examination of the blood showed fasting blood sugar, 448 and 422 mg. per cent; non-protein nitrogen, 145, 140, and 125 mg. per cent; CO_2-combining power, less than 4.5 meq. Roentgenograms showed a poorly defined cavity at the left apex where a small focus of calcification had been demonstrated in a previous year. No lesion was noted on the right. Physical signs of the lungs were not remarkable. He was given large amounts of fluid by clysis but urinary output was almost nil.

Gross Findings

There was edema of the ankles and right hand, and a crusted ulcer over the right wrist. The pleural spaces were obliterated by dense adhesions.

The right lung weighed 1,410 gm. and was covered by fibrinous adhesions. In the middle of the right upper lobe there was a firm, spherical, dark red area of consolidation, 5 cm. in diameter (Fig. 12), which contained thrombosed blood vessels. The remainder of the right lung was congested and edematous. The left lung weighed 1,900 gm. In the left upper lobe there was a cavity, 5 cm. in diameter, filled with yellow, friable, shreddy material and without a fibrous wall. Much of the upper lobe presented confluent lobular pneumonia. The remainder of the lung was edematous and congested.

The left kidney was reduced in size. The thyroid gland was symmetrically enlarged, with colloid nodules. The other organs, including the brain, were not remarkable.

An Aspergillus was cultured from the material within the cavity of the left lung.

Microscopic Findings

Microscopically, the consolidated region in the right upper lobe presented broad, branching hyphae within the walls and lumina of vessels and within the alveoli of adjacent tissue (Fig. 13). Neutrophilic response to the fungus in vessels was pronounced and islands of hyaline thrombotic material were forming. The hyphae varied from 6 to 25 μ in breadth, with occasional broader forms. There was minimal hemor-

rhage and no necrosis in the pulmonary tissue. Fungi were demonstrated in the middle and lower lobes, and three recently thrombosed vessels and wedge-shaped infarcts of small size were noted, as well as edema, congestion, and alveolar hemorrhage. The cavity of the left upper lobe contained mycelia. Some filaments were broad like those of the right upper lobe, while others were thin and beaded. Similar thin, beaded hyphae, with constricted, gram-positive segments, occurred in the purulent and fibrinous lobular pneumonia about the cavity. The "heads" of Aspergillus were seen in this material. No bacteria could be stained. The left lower lobe was edematous. Pus in a bronchus contained narrow hyphae like those just mentioned.

The kidneys presented severe chronic pyelonephritis with fibrosis, glomerulosclerosis, and hyaline arteriolosclerosis. The thyroid gland showed a nodular goiter.

Comment. This case was one of incidental mucormycosis of the right lung, of only 2 to 3 days' duration, occurring in a patient in diabetic acidosis. The pneumonia and cavitation of the left lung, due to a mixed fungus flora, appeared to be of 1 to several weeks' duration. The appearance of the fungus in the right lung, and its size, mode of branching, and spread through the walls of vessels were characteristic of mucormycosis, and were not compatible with appearances of Aspergillus in tissues. Diabetic coma and renal insufficiency were important causes of death, with the lobular pneumonia on the left contributing.

Case 6. Mucormycosis of Vessels of Lung, Spleen, and Liver Complicating Cutaneous Burns

B. T., a 3-year-old colored girl, received third degree burns, covering 75 per cent of her body surfaces, 13 days before death. She was taken immediately to the hospital and received fluid therapy. The urinary output increased to 1,685 cc. on the day before death and was 1,245 cc. on the day of death. Extensive penicillin therapy was given. No cortisone was administered. Rectal temperature had stabilized at 39° C. and on the day of death it was 40° C. The final clinical impression was death from extensive burns, with a question of pulmonary embolism.

Gross Findings

Third degree burns extended from the knees to the neck, including both upper extremities and the posterior aspect of the head and neck.

Soft, grayish brown material filled the vessels of the left upper lobe. The parenchyma was red and firmer than average. Focal regions of congestion were seen elsewhere in the lungs. Bronchi, trachea, and hilar nodes were not remarkable.

The heart was slightly dilated. Two small, irregular ulcers occurred in the duodenum, each measuring 1 by 0.5 cm. The spleen weighed 50

gm. and appeared normal. The left kidney weighed 140 gm. and the right, 95 gm. The brain was not remarkable.

Microscopic Findings

Microscopically, small vessels in lung, spleen, and liver contained numerous branching hyphae, 6 to 10 μ in thickness, which rarely showed cross septa. The blood in these vessels was coagulated to form recent hyaline thrombi. In other vessels there was aggregation of neutrophils without thrombosis. Hyphae extended through vessel walls and involved perivascular tissue, and neutrophils were conspicuous in some segments of arterial walls. The pulmonary tissue showed scattered foci of hemorrhage, edema, and atelectasis. The bronchi did not contain fungi.

Several minute vessels of the spleen contained hyphae. In the liver an artery 0.5 mm. in diameter contained numerous hyphae, a hyaline thrombus, and large numbers of neutrophils.

In the kidney, brownish casts were noted in the tubules, and fatty change in the renal epithelium of the convoluted tubules and loops of Henle.

Smears of the heart's blood showed no organisms. Cultures yielded a paracolon bacillus.

Comment. The intravascular mucormycotic infection may have been in part responsible for death as it had produced plugs in the pulmonary vessels.

Case 7. Disseminated Mucormycosis of Lungs, Kidney, Liver, and Large Vessels with Giant Cell Reaction

D. M., a 3-year-old white boy, had an illness of 3 to 4 months' duration, leading to death. No bacterial causative agent had been identified. A yeast had been recovered from pleural fluid on one occasion.

Microscopic Findings

Microscopically, the pulmonary vessels contained peripherally located, branching, non-septate hyphae, 8 to 12 μ thick, with conglomerations of fragmented nuclei and recent thrombi. Regions of coagulation necrosis, interpreted as infarcts, contained the fungus as well as neutrophils, minute abscesses, macrophages, fibroblasts, and giant cells. Both abscesses and giant cells contained hyphae (Fig. 14). Beyond the infarcted regions there was chronic interstitial pneumonia, bronchitis, and chronic pleurisy, without fungus. There were gram-positive cocci in chains in one area of lung. The mycosis in the lung was judged to be of approximately 1 month's duration.

A large artery, of uncertain location in the body, presented severe necrotizing arteritis, with numerous hyphae in the wall, occasionally within giant cells. Nuclear débris and eosinophils were conspicuous.

Hyphae were present, with giant cell response, on the surface and beneath the capsule of the liver, and in a large recent infarct of the kidney, which also contained bacterial masses.

Comment. This was the only case with giant cell response.

Discussion

Predisposing Factors

Diabetes mellitus was an antecedent disease in 4 of the cases of pulmonary mucormycosis (Table I). Diabetes has also been an important antecedent disease in cases of the cerebral form of mucormycosis. This predisposition to cerebral mucormycosis recently has been demonstrated experimentally in alloxan-induced diabetes in rabbits given spores of Rhizopus intranasally.[9] Diabetes predisposes to many infections, and mucormycosis appears to be no exception.

Leukemia was the antecedent disease in 2 cases. While leukemia also predisposes to infection, there is the possibility that chemotherapy, antibiotics, or cortisone may have increased the susceptibility of the patients to mucormycosis. The same might be suggested for the case of multiple myeloma. In the 3 remaining cases in which burns, infantile diarrhea, and undiagnosed infection were present, there is the possibility that antibiotic drugs suppressed bacteria and favored the mycosis.

From the data of Table I, there is no reason to suggest that age, race, or sex are important etiologic factors in themselves. Geographically, all of these cases have occurred in the United States. A case of cerebral mucormycosis has been reported from England.[3] Reference will be made later to cases of pulmonary mucormycosis reported in the older German literature.

The Fungus

The fungus in the lesions of these cases of pulmonary mucormycosis had broad, branching, coenocytic (non-septate) hyphae, from 4 to 20 μ in width and up to 200 μ in length. No sporangia or spores were found.

In the tissues the fungus was demonstrated well by ordinary hematoxylin and eosin staining (Figs. 3, 6, 8, 9, 10, 11, 13, and 14), and even better by iron hematoxylin staining (Fig. 4). Staining by Gram's method or by the periodic acid-Schiff method did not prove to be superior. An indigocarmine stain,* based on a suggestion by Christian-

* Baker, R. D., and Smith, Josephine, April, 1955.

sen[10,11] for use on mucormycotic animal tissues, and perfected in this study, gave good results (Fig. 5).

Indigocarmine Stain for Mucormycosis

1. Rinse deparaffinized sections in acidified water (0.5% acetic acid)
2. Stain 5 to 10 minutes with Goodpasture's aniline carbol fuchsin (30% alcohol, 100 cc.; basic fuchsin, 0.59 gm.; aniline oil, 1 cc.; phenol, 1 gm.)
3. Rinse well in running water
4. Rinse in acidified water
5. Stain 5 to 10 minutes with picro-indigocarmine (dissolve 0.25 gm. of indigocarmine in 100 cc. of saturated aqueous picric acid)
6. Rinse, rapidly, in acidified water
7. Dehydrate, rapidly, in 95% alcohol and two changes of absolute alcohol
8. Clear in xylene and mount in Permount

Results: Nuclei red, fungus and collagen blue-green, background yellow to colorless.

Notes: (a) Tissue may be fixed with any good fixative.

(b) Mayer's carmalum, 5 to 10 minutes, may be substituted for aniline carbol fuchsin if desired. (Dissolve 1 gm. carminic acid and 10 gm. ammonia alum in 200 cc. of distilled water, with heat, if necessary. Filter and add 1 cc. of formalin as a preservative. Tissue stained should not be alkaline.)

(c) Sections may be previously mordanted in aqueous picric acid or 5% ammonia alum at 60° C. for about 1 hour to increase nuclear staining if carmalum is used.

Cross septa occurred rarely in these coenocytic or non-septate organisms. Angulations of hyphae and other artefactitious circumstances were noted which only simulated septa and could be excluded by careful focusing. In other instances, it was necessary to accept the presence of occasional cross septa. Another peculiarity consisted of unusually thick or thin hyphae. Where the fungus appeared to be growing luxuriantly, it was often thick. In case 5, broad, slightly dilated regions were noted, up to 35 μ in diameter. In regions where infarction had occurred or where the tissues were necrotic, as in cases 1 and 7, hyphae were shrunken and irregular as though the central material had been lost. These hyphae may have been dead and may have developed new osmotic pressures. Another feature, that of length, was conditioned by the thickness of ordinary sections. When extremely thick sections were cut, for example, up to 200 μ, hyphae were seen which extended for much greater distances than the 200 μ mentioned in the descriptions. The fungus in the lesions of the lung had the same appearances as those of the lesions of cerebral mucormycosis, and there was the same tendency to invade vessels. Case 4 displayed both pulmonary and cerebral lesions, and aided in establishing the unity of the two forms of the disease.

In tissues the organisms of aspergillosis may simulate those of mucormycosis and may also invade blood vessels in a similar fashion. However, it is possible to identify aspergillosis in tissues because Aspergillus has numerous cross septa, is narrower, branches at more

acute angles and in brush-like fashion, and tends to produce abscesses.

Although the fungus of mucormycosis was not grown in culture from any of the cases of Table I, the cases are presented as examples of mucormycosis on the basis of the appearances of the fungus in the lesions, and the tendency to produce thrombosis and infarction.

There is direct evidence to suggest that Rhizopus may be the cause of the pulmonary mucormycosis which we have described, because a fungus of this genus has been cultured from 2 cases of cerebral and orbital mucormycosis in which the appearance of the fungus in tissue was the same as that in our cases. In the case of palatal and orbital mucormycosis, described by Harris,[6] *Rhizopus arrhyzus* was cultured from the palatal ulcer and biopsies of the ulcer showed hyphae in tissues and in blood vessels similar to those in our pulmonary cases. In a recent cerebral case in Georgia,[5] *R. oryzae* was cultured from an ethmoid sinus, while the cerebral lesions were like the lesions of our pulmonary cases with respect to structure of fungus and involvement of blood vessels. Moreover, intravenous inoculation of spores from these two species of Rhizopus into rabbits produced renal lesions containing hyphae similar to the hyphae in the pulmonary lesions of our human cases.[12] While species of Rhizopus may be the cause of pulmonary mucormycosis, other Phycomycetes, including species of Mucor and Absidia, must not be excluded for the present. Some of the older German cases of mucormycosis were caused by true Mucors because characteristic sporangia were seen in tissues and because, in one case, a Mucor was cultured from a lesion.

Among 9 cases of mucormycosis in swine, a Rhizopus was cultured in one and *Absidia ramosa* (*Vuillemin*) *Leudner* in 8 cases.[10,13]

Davis and co-workers[14] recovered a fungus from a case of bovine mucormycosis, which was isolated in culture and identified as *Lichtheimia* (*Mucor*) *corymbifera*. The culture and tissue sections of the lesion were sent to me. Dr. N. F. Conant agreed that the culture was a Mucor. In sections the lesion was similar to those of our cases of pulmonary mucormycosis, but there were differences. In the slide from the bovine lesion there were hyphae with bulbous ends, and with thickenings along hyphae. There were curious forms in which bulbous heads seemed to come together in thin hyphae (4 μ thick). These appearances were different from those of the human cases and suggested that cases due to true Mucors may have characteristic hyphae in sections.

Both Rhizopus and Mucor belong to the Phycomycetes. Rhizopus is characterized by rhizoids, or root-like structures, in culture, and by terminal sporangia borne on non-branched sporangiophores arising in

fascicles at the point of rhizoid formation. Mucor has no rhizoids, but produces numerous sporangiophores of unequal length, which branch irregularly and bear terminal sporangia.[15] Since Rhizopus and Mucor both belong to the Mucoraceae, the term mucormycosis continues to be appropriate to designate infection by either Rhizopus or Mucor.

The exact determination of the causative fungus of human pulmonary mucormycosis must await cases with cultural studies.

Pathogenesis of Pulmonary Mucormycosis

The respiratory tract appeared to be the portal of entry of the fungus in the majority of cases (cases 1, 2, 3, 5, 8, and 10). Presumably the fungus was aspirated into the bronchi and multiplied within them. In favor of this view was the presence of rich intrabronchial fungus growth in cases 1, 2, and 3. The fungus was observed in sections to be permeating the bronchial walls (Fig. 8). The accompanying bacterial flora in bronchi (cases 2 and 3) may have favored the penetration of the bronchial walls. Hilar and peribronchial tissues showed hyphae in sections (case 1). Infection of arterial walls and thromboarteritis apparently followed, with the production of infarcts. While this is a peculiar and unorthodox form of spread of an infectious agent, the morphologic studies suggest it strongly.

Vascular spread of the pulmonary infection, especially by arteries, was highly important, and thrombo-arteritis was noted in the majority of cases. Thrombophlebitis was demonstrated in addition to thromboarteritis in cases 1 and 3, and it may have been the chief vascular involvement in case 8. Permeation of lymphatics by the fungus was observed (Fig. 11). Hilar lymph nodes were invaded by hyphae (cases 1 and 4), apparently from the general hilar involvement as much as from drainage from pulmonary lymphatics.

Dissemination of the mycotic infection from the lungs to other parts of the body did not appear to be an important complication, except possibly in case 10.

Hematogenous spread to the lungs from primary orbital or cerebral mucormycosis appeared to have occurred in cases 4 and 9. The orbital and cerebral forms arise from infection of the nasal and paranasal sinuses.[2,5]

The third possibility of source of the mycosis is from denuded cutaneous surfaces. For example, in case 6 there were extensive cutaneous burns with intravascular mycosis of lungs, liver, and spleen and with no demonstrable fungus in bronchi.

The penetration of tough arterial walls by the hyphae of this fungus

is a unique phenomenon, difficult to understand or explain. The walls were viable in many instances, judging from the continuing nuclear staining, and the fungus was apparently invading living rather than necrotic tissue.

Mixed infections were noted in 5 cases. In 2 cases there were bacteria in bronchi, gram-positive cocci in one and gram-positive rods in the other. In case 2 there were masses of cocci in a softened region in the center of the infarct and the patient had had stomatitis due to Candida. In case 7 there were gram-positive cocci in one area of a lung and bacterial masses in the kidney. Yeast cells occurred in the bronchus of one case, while Aspergillus and other fungi occurred in the opposite lung of case 5. These mixed infections probably indicate the lack of resistance of the body to infections in general; or, they may have acted in a synergistic manner to permit mucormycosis to develop; or, the bacteria may have been inhibited by antibiotics, failing to invade, while the organisms of mucormycosis were not inhibited.

Course of Pulmonary Mucormycosis

Pulmonary mucormycosis gave clinical evidence of infection in most of the cases, although the nature of the infection was not suspected. In case 1 the onset was sudden, with severe chest pain, pleural friction rub, and the production of bloody sputum. This probably coincided with the development of the infarct. In several cases fever and leukocytosis were present and there was an increase in the number of immature neutrophils during the course of the infection. In case 3, for example, the white blood cell count was 23,550 per cmm. with 94 per cent neutrophils. Roentgenograms showed pulmonary consolidation (Figs. 1 and 7).

The duration of the mycosis varied from 3 to 30 days.

Pulmonary mucormycosis was an incidental finding at necropsy in several of the cases, and not suspected clinically. In 6 cases, pulmonary mucormycosis appeared to be the main cause of death.

The lesions of pulmonary mucormycosis did not respond to antibacterial agents as bacterial pneumonia would have done. No specific agent is known to combat the fungus, although iodides may be used. Desensitization of the patient to his own infecting organisms may be of value. Regulation of diabetes was apparently the chief factor in the survival of the patient with palatal and orbital mucormycosis reported by Harris.[6] Clinical cases of pulmonary mucormycosis have been reported in which the patient recovered.[16-18] The diagnoses of these cases have been based on culturing the organism from sputum;

and this in itself is not sufficient to establish the diagnosis of pulmonary mucormycosis, since these organisms are common contaminants.

The Lesions of Pulmonary Mucormycosis

The lesions of pulmonary mucormycosis were a combination of mild acute inflammation and of infarction due to vascular obstruction. Neutrophilic response to the presence of the hyphae in pulmonary alveoli and within vessels was present. Fibrin, hemorrhage, and edema were observed frequently in situations in which it was not clear whether inflammation or ischemia was the more important causative factor. One case (case 7) was characterized by giant cell response, phagocytosis of hyphae, and eosinophils and lymphocytes. Coagulation or "caseous" necrosis was noted repeatedly, in most cases simply representing infarction.

The hyphae in renal lesions of intravenously inoculated Rhizopus in rabbits were surrounded by a zone of intense polymorphonuclear cell response, indicating the primary neutrophilic reaction to the fungus.

Mucormycosis of Older Literature

The older German literature contains numerous reports of pulmonary mucormycosis. Those which appeared to fulfill the criteria of having broad, branching, non-septate hyphae in tissue are gathered together in Table II. They are not included in Table I, because they were not as well documented with photomicrographs as the more recent cases, and because hyphae frequently presented club-shaped excrescences, unlike the fungus of our series. Sporangia were frequently present in tissue and an affinity for vessels was less regular. Four of the 6 acceptable cases were instances of incidental discovery at necropsy of small subpleural nodules. In one, the pulmonary lesions were part of disseminated mucormycosis and in one, it was a secondary infection of a tuberculous cavity. In all but one of the cases there was a predisposing condition. These early German reports appear acceptable as instances of mucormycosis, but they may well be due to other forms of Phycomycetes than those in Table I.

Doubtful Reported Cases

In a recent case, reported as pulmonary mucormycosis,[24] a Mucor was cultured, but hyphae obtained directly from the lesion presented frequent septa with constrictions at these points and do not appear to be coenocytic. Several other cases are considered doubtful as examples of mucormycosis because of inadequate identification of the fungus in tissues.[25-27]

TABLE II

Cases of Pulmonary Mucormycosis from Older Literature

Author	Year	Age	Sex	Gross	Microscopic	Significance of pulmonary mucormycosis
Fürbringer[19]	1876	66	M	2 subpleural, walnut-sized, hemorrhagic foci, right upper lobe	Hyphae 4–20 μ thick with club-shaped projections; sporangia with columellae in tissues	Incidental to gastric carcinoma with widespread metastases
Fürbringer[19]	1876	31	M	3 apical, walnut-sized, greasy, yellow nodules	Same as the first case; both called Mucor	Incidental to severe "gastro-intestinal catarrh"
Paltauf[20]	1885	52	M	Numerous round, dark foci, 1.5 cm. in diameter	Hyphae 3–6 μ thick with warty excrescences; sporangia in lesions	Hematogenous pulmonary lesions part of disseminated mucormycosis of 2½ weeks' duration, originating in intestines
Podack[21]	1899	39	M	Subpleural nodules	Hyphae with terminal enlargements; spores with off-shoots; sporangia probably present; hyphae permeated vessels; bacteria in lesions	Incidental to other disease
Lang and Grubauer[22]	1923	56	F	Tuberculous pulmonary cavity	Broad hyphae in vessel walls of lining of cavity; thrombi; sporangia; tubercle bacilli; *Mucor corymbifer* cultured	Death due to mucormycotic and coccal pneumonia
Wätjen[23]	1929	34	F	Subpleural cavities in right lower lobe	Fungus in hemorrhagic infarcts; sporangia; fungus thought to be *Mucor corymbifer* in tissues	Incidental finding; death due to sarcoma

Summary

Pulmonary mucormycosis appears to be a new disease in the United States, and one of increasing frequency, probably due to the use of antibiotics which suppress the growth of bacteria and thereby permit the invasion of fungi. The possible rôle of cortisone, ACTH, and antileukemic drugs in favoring invasion of the fungus is suggested also.

Six cases of pulmonary mucormycosis are reported. With the 4 cases already in the recent literature, this brings the total to 10. The feature common to these cases was the presence of broad (4 to 20 μ thick), branching, predominantly non-septate hyphae in the lesions. The fungus penetrated the walls of arteries, veins, and lymphatics and produced thrombosis and infarction. Organisms spread also in the bronchi and alveoli, producing mycotic bronchitis and pneumonia. The hilar structures and pleura were invaded, and the diaphragm was infected in one case. Organisms were seen easily in hematoxylin and eosin stained sections.

The mycosis varied in duration from 3 to 30 days, and presented the clinical and roentgenographic features of pneumonia and pulmonary infarction, with fever and leukocytosis.

Predisposing diseases were diabetes mellitus in 4 cases, leukemia in 2, multiple myeloma in one, cutaneous burns in one, infantile diarrhea in one, and there was no known predisposing disease in one case. The mucormycosis developed as an intercurrent or terminal infection in these diseases.

Pulmonary mucormycosis has been observed in North Carolina, South Carolina, Texas, Arizona, and Massachusetts.

Most of the infections developed from inhalation of ubiquitous common Phycomycete contaminants, possibly Rhizopus, with the production of mycotic bronchitis and pneumonia, thrombosis, and infarction. Some of the infections were hematogenous, coming from cerebral mucormycosis.

While cultures of the pulmonary lesions for fungi were not obtained, it appeared probable that at least some of the cases were due to Rhizopus, since the histologic appearances were identical with those of Rhizopus as it infects the brain and palate of cases of mucormycosis of those regions.

The organisms in cases of pulmonary mucormycosis reported in the older German literature had forms of hyphal growth and sporangial production in tissues not noted in the cases from this country. Some of these foreign cases apparently were produced by true Mucors.

The diagnosis of mucormycosis is made by the demonstration of fungi in lesions or in material from lesions. Culturing of Phycomycetes

from sputum is not diagnostic of mucormycosis because these organisms are common contaminants.

Therapy of mucormycosis is unsatisfactory, since no chemical agent is available which has a specific effect on the fungus and which the patient can tolerate. Iodides and desensitization may be used. Treatment of the predisposing condition, such as regulation of uncontrolled diabetes, is of first importance.

In reporting new cases of pulmonary mucormycosis, I am greatly indebted to pathologists who have sent me material or have aided in the preparation of protocols, as follows: cases 1, 5, and 6, Drs. Albert Sion, J. O. Wynn, and John R. Archdeacon of the Department of Pathology, Duke University School of Medicine, Durham, N.C.; case 2, Dr. George Vennart, Department of Pathology, University of North Carolina, Chapel Hill, N.C.; case 3, Dr. Hunter W. May, Self Memorial Hospital, Greenwood, S.C.; case 4, Dr. A. O. Severance. Baptist Memorial Hospital, San Antonio, Texas; case 7, Dr. O. O. Williams, St. Joseph's Hospital, Phoenix, Ariz.

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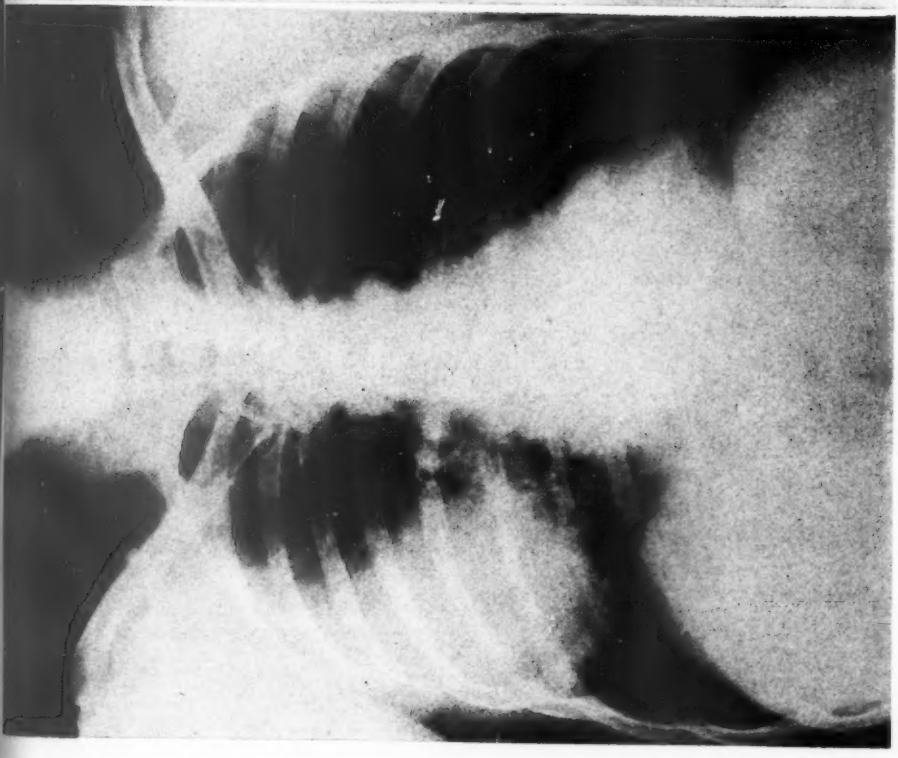
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Case 1. Roentgenogram of chest taken 11 days before death, showing mucormycotic infarct of right lung.

FIG. 2. Case 1. Right lung showing plug of fungus in bronchus to upper lobe, thrombi in pulmonary arteries, infarct involving entire lung except apex and base.

2



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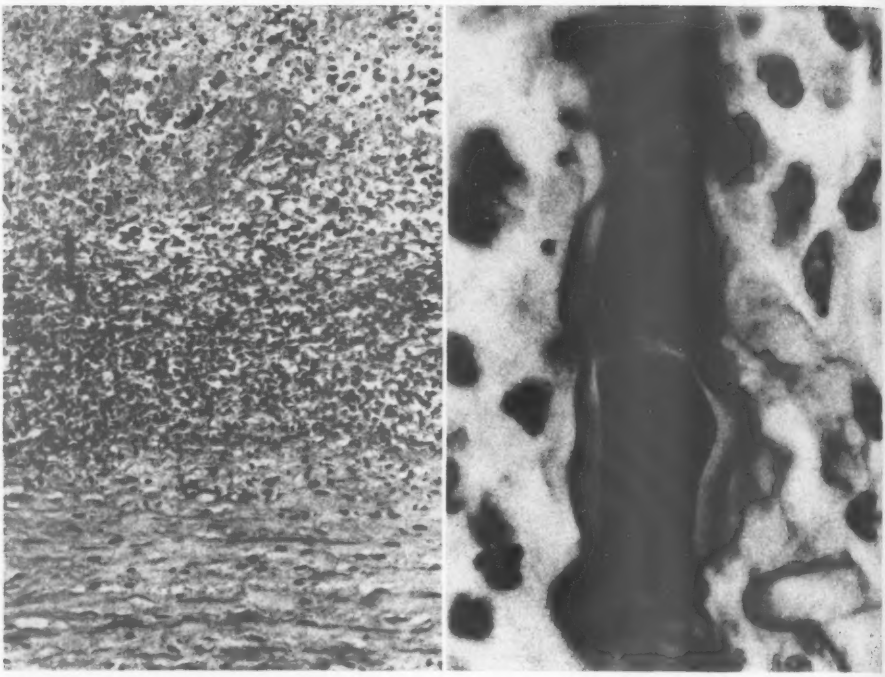

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FIG. 3. Case 1. Right pulmonary artery showing media of artery at the bottom; neutrophilic infiltration at periphery of thrombus, in the middle; and hyaline thrombus with fungus hyphae, at the top. Hematoxylin and eosin stain. × 200.

FIG. 4. Case 1. Segment of hypha in the wall of a pulmonary blood vessel. Iron hematoxylin and eosin stain. × 1,487.

FIG. 5. Case 1. Branching, non-septate hyphae in diaphragm. Indigocarmine-picric acid stain. × 200.

FIG. 6. Case 1. Hyphae in diaphragm, with swollen and necrotic muscle fibers and acute myositis. Hematoxylin and eosin stain. × 409.

FIG. 7. Case 3. Roentgenogram of left hilar and basal lesion of chest taken the day before death.

FIG. 8. Case 3. Hyphae in lumen of bronchus. The hyphae in the wall are seen as rounded spaces in some instances. Subacute bronchitis with loss of epithelium. Hematoxylin and eosin stain. × 200.

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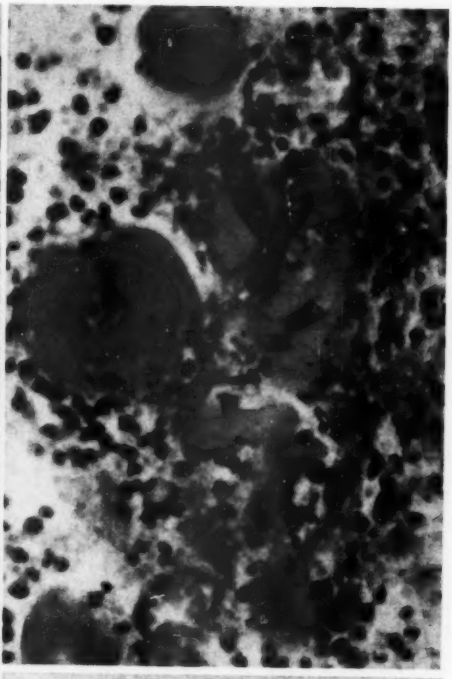

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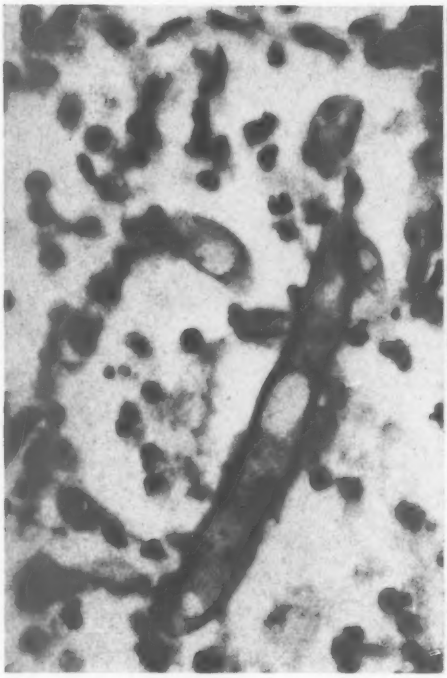

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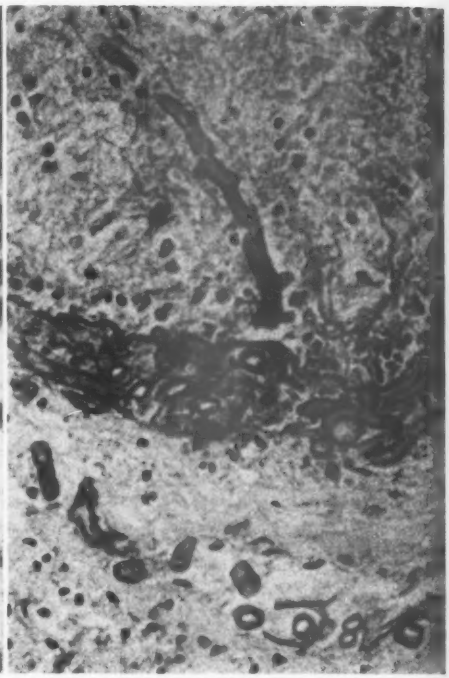

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FIG. 9. Case 4. Hypha in alveoli of lung, with neutrophilic response. Hematoxylin and eosin stain. × 700.

FIG. 10. Case 4. Hyphae in wall of pulmonary vessel (mid-field), outside the vessel (at bottom), and extending into lumen (at top). Hematoxylin and eosin stain. × 425.

FIG. 11. Case 4. Hyphae in perivascular lymphatics of lung. Hematoxylin and eosin stain. × 132.

FIG. 12. Case 5. Rounded region of congestion in right upper lobe due to mycotic thrombosis of blood vessels.

FIG. 13. Case 5. Hyphae in wall (below) and lumen (above) of blood vessel from gross lesion shown in Figure 12. Hematoxylin and eosin stain. × 132.

FIG. 14. Case 7. Giant cell response to a hypha. × 700.

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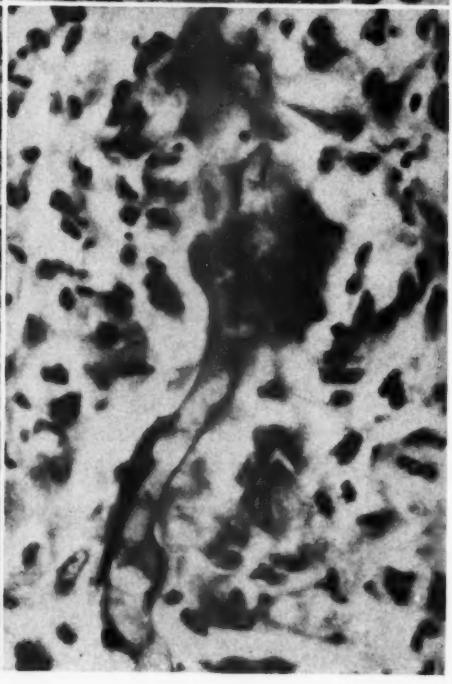

14

THE HISTOPATHOLOGY OF BROWN FAT IN EXPERIMENTAL POLIOMYELITIS *

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Through the agency of cortisone administration, experimentally induced poliomyelitis in the Syrian hamster is dramatically enhanced.[1,2] This augmentation is accompanied by reproducible histologic lesions in striated muscle[3] and fat.[4] The time of emergence and distribution of these extraneural foci parallels the levels of viral infectivity of such tissues, thus corroborating a relationship between the pathogen and observed histopathologic change.[5,6]

Studies designed to clarify the pattern of involvement following various routes of administration of poliomyelitis virus revealed a singular distribution within adipose tissue. The regions of fat necrosis reflecting virus activity were confined exclusively to that form of fatty tissue which has been designated variously by anatomists as brown fat, hibernating fat, glandular fat, or embryonal fat. White fat was histologically unaffected by the extensively disseminated viral infection.

MATERIALS AND METHODS

Male Syrian hamsters, weighing between 25 and 35 gm., were used, except when otherwise specified. Poliomyelitis virus (strain MEF_1) was kindly supplied by Dr. Peter K. Olitsky and maintained in this laboratory by serial intracerebral mouse passages. A pooled emulsion of such mouse brains (LD_{50} mouse titer, $10^{-3.7}$), refrigerated at –30° C., was used. Two other type 2 poliomyelitis strains (Lansing, Yale-SK), available as mouse brain emulsions, also were used. A rodent encephalitis virus (Columbia-SK), obtained from Dr. C. W. Jungeblut, was used for comparative experiments. Three strains of type 1 poliomyelitis virus—MacMahon, Wisconsin, and Brabyn (isolated from human stool and typed by Dr. Robert Ward)—were used in monkey experiments.

As outlined in Table I, 509 male hamsters were divided into experimental and control groups. Animals from all groups were sacrificed daily for purposes of histologic investigation as well as for the determination of infectivity levels of the virus in the tissues under study. Cortisone was administered in dosages described in Table I.

Animals were sacrificed with chloroform and necropsied immedi-

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TABLE I

Lesions Produced by Various Strains of Poliomyelitis and Columbia-SK Viruses

Virus	Dilution	Route	Animal	No. used	Cortisone	Paralysis	Myositis	Brown fat necrosis
						%		
MEF_1	1:20	I.C.	Hamster	40	5 mg. simultaneously with virus	100	++	++
MEF_1	1:20	I.C.	Hamster	40	None	45	−	−
MEF_1	1:20	I.P.	Hamster	40	3 mg. 4×; 0, 5, 19, 5 hrs.	100	+++	+++
MEF_1	1:20	I.P.	Hamster	40	None	0*	−	−
MEF_1	1:20	I.M.	Hamster	60	3 mg. 4×; 0, 5, 19, 5 hrs.	100	++++	++++
MEF_1	1:20	I.M.	Hamster	20	None	0	−	−
MEF_1	1:20	S.C.	Hamster	20	3 mg. 4×; 0, 5, 19, 5 hrs.	100	++	+++
MEF_1	1:20	S.C.	Hamster	10	None	0	−	−
MEF_1	1:100	I.P.	White mouse	50	2 mg. 2×; 0, 24 hrs.	4	±	±
MEF_1	1:100	I.P.	White mouse	20	None	0	−	−
MEF_1	1:100	I.M.	White mouse	20	2 mg. 2×; 0, 24 hrs.	15	−	±
MEF_1	1:100	I.M.	White mouse	10	None	0	−	−
Yale–SK	1:50	I.M.	Hamster	6	3 mg. 4×; 0, 5, 19, 5 hrs.	83	++	++++
Yale–SK	1:50	I.M.	Hamster	6	None	0	−	−
Lansing	1:20	I.P.	Hamster	37	3 mg. 4×; 0, 5, 19, 5 hrs.	40	+	+
Lansing	1:20	I.P.	Hamster	20	None	0	−	−
Columbia–SK	1:20	I.C.	Hamster	10	5 mg. simultaneously with virus	100	+++	−
Columbia–SK	1:20	I.C.	Hamster	10	None	100	++	−
Columbia–SK	1:20	I.M.	Hamster	10	5 mg. simultaneously with virus	100	+++	−
Columbia–SK	1:20	I.M.	Hamster	10	None	100	++	−
Columbia–SK	1:20	S.C.	Hamster	10	5 mg. simultaneously with virus	100	+++	−
Columbia–SK	1:20	S.C.	Hamster	10	None	100	++	−
None			Hamster	110	3 mg. 4×; 0, 5, 19, 5 hrs.	0	−†	−‡

I.C. = Intracerebral. I.P. = Intraperitoneal. I.M. = Intramuscular. S.C. = Subcutaneous.

* While no animals in this category have shown clinical evidence of infection, examination of the spinal cords of a more comprehensive group of hamsters inoculated with the virus intraperitoneally has disclosed very rare instances of focal poliomyelitis; less than 1% of hamsters, so inoculated, presented such subclinical lesions.

† Previous studies on viral myositis included a large control series of hamsters treated only with cortisone.[8] A very rare hamster showed some spotty muscle changes of an inflammatory nature, possibly due to the activation by cortisone of some latent pathogen.

‡ Steatopathy of brown fat, of the type described in this communication, was never seen in animals receiving cortisone alone. Some control animals occasionally showed old calcified granulomatous foci.

ately. Tissues were preserved in 10 per cent buffered formalin, Bouin's solution, chilled acetone, and lemon juice. Staining procedures included hematoxylin and eosin, Mallory's phosphotungstic acid-hematoxylin, MacManus' periodic acid-leukofuchsin, Laidlaw's reticulum stain, Baker's acid hematein, Bacchus' ascorbic acid technique,[6a] Gomori's alkaline phosphatase and esterase stains, Sudan black, Sudan IV, Ranvier's nerve ending stain, von Kossa's method, and Schultz's procedure for cholesterol.

Findings

Fat lesions, of a type to be described, were confined to experimental animals receiving both cortisone and poliomyelitis virus. These foci of necrosis, as in the case of the co-existent specific myositis,[3] preceded the establishment of spinal cord involvement, and emerged during the preparalytic phase of the potentiated disease. While lesions within brown fat were evident ubiquitously at the height of the disease, a regional pattern of distribution was evident.

Distribution and Histologic Features of Brown Fat

Brown fat has been recognized for centuries as a distinctive form of lipid-containing connective tissue, found in almost all mammalian species, but more prominent in animals characterized by seasonal cycles of dormancy. Its anatomical distribution has been described extensively in the hedgehog, marmot, bat, and various other rodents.[7,8] In the marmot, this peculiar form of fat has been recorded as found in a large mass near the thymus with extensions into the intermuscular septa of the neck with further slender prolongations dorsal to the clavicles and frequently continuous with prominent axillary lobes. Brown fat is discernible also in the thorax, paralleling the sympathetic nervous system chains and the mammary arteries. Discrete zones of brown fat are seen in the interscapular and perirenal areas. The inguinal fat occasionally contains a small amount of this tissue.

For other species, the distribution of brown fat is described as essentially similar. Three principal zones are recognized: interscapular, with prolongations into dorsal and cervical musculature and axillae; thoracic, about the thymus and parallel to the thoracic aorta; and abdominal, enclosing the adrenal glands and about the renal vessels.

The location of brown fat in the Syrian hamster does not differ materially from the previously recorded anatomical surveys of other Rodentia (Fig. 1). Some quantitative distinctions peculiar to the hamster merit attention, however. The major depot of this specialized tissue is ventral to, and between the scapulae, in the form of well demarcated, tawny-orange lobes. The most prominent of these masses is located in the midsagittal dorsum, between the scapulae, extending from the lower cervical level to the midthoracic plane. Clearly distinguishable, lobulated masses of brown fat extend beneath the scapulae, and, in variable continuity, occupy the axillary apices. Digital extensions are observed rostrally between the posterior cervical muscles. Only minute amounts of brown fat are apparent around the thymus and thyroid gland. Further tissue of this nature surrounds the sympathetic nervous chain in its thoracic course. The adrenal glands are sheathed by minute crescent-shaped masses of brown fat and similar masses are located in the soft tissue of the renal hilum in close association with the ureter. In the average 40 gm. hamster, brown fat by weight is distributed as follows: interscapular, 19.2 per cent; subscapular (bilateral), 28.8 per cent; axillary (bilateral), 24 per cent; periadrenal (bilateral), 1.9 per cent; peri-

changes of an inflammatory nature, possibly due to the activation by cortisone of some latent pathogen.
1 Steatopathy of brown fat, of the type described in this communication, was never seen in animals receiving cortisone alone. Some control animals occasionally showed old calcified granulomatous foci.

thymic, 2.2 per cent; thoracic paravertebral (bilateral), 5.4 per cent; anterior cervical intermuscular (bilateral), 3.4 per cent; and posterior cervical intermuscular (bilateral), 15.1 per cent.

In monkeys, the principal site of brown fat is noted in the axillae in the form of adjoining lobules. A small amount of this tissue is found also between the posterior and lateral cervical muscles, as well as in the scapular area. Minute amounts are detected in the thoracic paravertebral region and in the soft tissues encasing the adrenal glands.[9] Preliminary studies, to be recorded at a later date, disclose the presence of brown fat in humans. The tissue is found in approximately the same regions as in simians.

In contrast to the unconfined nature of white fat, the brown fat lobule is seen as a delimited structural entity. It is invariably encapsulated by a thin fibrous sheath. The microscopic appearance of these lobules is uniformly monocellular (Fig. 2). Only rarely are white fat cells present within the borders of such lobules. The brown fat cell is quite distinctive. It is smaller than the white fat cell, measuring under normal circumstances about 23 μ, as compared to 41 μ for the white fat cell. Brown fat cells are usually round or polygonal, with centrally situated nuclei (Fig. 3). The cytoplasm contains a visible amount of eosinophilic material which is not removed by routine histologic solvents. Mingled with this intracellular residuum are numerous isolated lipid-filled vacuoles, which prompted the earlier designation of the brown fat cells as "polylocular cells."[8] Surrounding each brown fat cell is a rich capillary plexus which is not demonstrable within white fat. The constituent cytoplasmic lipids differ in the two types of fat cells. Some sudanophilic material persists in brown fat cytoplasm after cold alcohol and cold acetone extraction. The entire cytoplasm of the white fat cell and the small cytoplasmic vacuoles of the brown fat cell stain in a similar fashion and react similarly to differential extraction procedures. The meshwork surrounding the polylocular vacuoles of brown fat cells, however, appears to contain sudanophilic substances which have no counterpart in white fat. When Baker's acid hematein stain is used, the brown fat cytoplasm assumes a blue-black granularity which can be removed by preliminary pyridine extraction. A negative reaction with this staining procedure is obtained with white fat. The triad of sudanophilia following alcohol immersion, blue-black staining with the Baker procedure, and removal of this reaction by pyridine indicates the presence of phospholipid. There are further distinctions between the two forms of fat. A perceptible amount of ascorbic acid is demonstrable in brown fat by Bacchus' technique. Gomori's alkaline phosphatase procedure discloses some positive reaction in brown fat cytoplasm and nucleus, but not in adult fat. A considerable amount of esterase is localized within brown fat cytoplasm also. No appreciable difference can be seen in the number of traversing nerve fibers within the two respective forms of fat tissue as determinded by the Ranvier technique for nerve endings.

Brown fat can thus be distinguished from white fat by a unique anatomical distribution, tawny-orange coloration, the presence of organoid lobulation, a morphologically distinct cell architecture, an extensive and intimate vascularity, and increased quantities of demonstrable phospholipid, ascorbic acid, alkaline phosphatase, and esterase.

Pathologic Changes of Brown Fat Caused by Poliomyelitis (MEF_1) Virus

The initially observed changes were intracellular, and consisted of a smudginess and hypereosinophilia of the brown fat cytoplasm. The delicate, interlacing, cytoplasmic filaments were replaced by coarse,

swollen, often granular fragments which migrated to the peripheral cell membranes (Fig. 4). The nucleus, previously located centrally, became pyknotic and was retracted centrifugally. At this stage of cellular degradation, the central zone of the cell was cleared of detritus and structure. The residual cytoplasm was reduced to crescent-shaped basophilic masses of necrotic, granular substance surrounding rapidly disintegrating nuclear fragments (Fig. 5). The products of necrobiosis often formed a beaded pattern along the inner cell membrane. Intermingled between the larger particles of intracellular débris were small, spherical globules which measured 0.8 to 1.2 μ in diameter (Fig. 6). The superimposed basophilism became apparent very shortly after degeneration of fat began and coincided with calcific precipitation within the necrotic material. Calcium deposition, as shown by the von Kossa technique, occurred only in loci of intracellular necrobiosis. It was a constantly observed feature and was demonstrable within 24 hours after the onset of cellular breakdown (Fig. 7). The minute granules also reacted positively for calcium with this procedure. At this stage, and in all succeeding phases of brown fat necrosis, the intracellular fragments and granules were strongly sudanophilic. Negative reactions were obtained with the Feulgen (thymonucleic acid) technique, Gomori stain for iron, and Gram stain for bacteria. The MacManus periodic acid-leukofuchsin stain imparted a distinct violet-red color to the degenerating intracellular material. This reaction was not abolished by previous incubation in 0.1 per cent diastase (3 hours, 37° C.), 0.1 per cent hyaluronidase (3 hours, 37° C.), or hot acetone (17 hours, 58° C.). Occasionally, clear vacuoles were perceived within a region of MacManus-positive matter, often containing a minute granule of intensely positive staining substance. Sections prepared by the Gomori procedure for the determination of alkaline phosphatase activity showed a strong reaction within necrotic fragments and spherules. Thirty-six hours after the onset of brown fat necrosis, the involved cell was reduced to an incomplete and obscured cell membrane surrounding confluent, arc-shaped masses of basophilic, calcified material. Some of the minute granules escaped into the intercellular spaces. Secondary inflammatory reaction did not play a significant rôle in the fully expressed picture of brown fat steatopathy caused by the MEF_1 virus in hamsters treated with cortisone. In animals surviving 6 days, a retarded and scanty response was observable within the interstitium (Fig. 8), and occasionally a granulomatous reaction was seen (Fig. 9). During the time limits of the experiments, no evidence of tissue reparation was recorded.

The differential lipotropism exhibited by the MEF_1 virus can best be appreciated by histologic observation of regions where both forms

of fat were in intimate association. The periadrenal brown fat, while a discrete structural entity, was largely enclosed by white adipose tissue. Only a thin fibrous septum intervened between the two. The microscopic lesions resulting from MEF_1 virus activity were totally restricted to the brown fat tissue. At most, a few lymphocytes were seen in white fat immediately bordering the brown fat lobules. The phenomenon of selective involvement could even be discerned grossly in the rare hamsters that survived beyond the fifth day of viral infection. In the normal hamster, the interscapular and periadrenal brown fat bodies were easily distinguishable grossly, since the amount of surrounding white fat was minimal. They appeared as circumscribed, lobulated, brownish orange masses which were somewhat more opaque than white fat tissue. The brown fat structures within the cervical, mediastinal, axillary, and humeral regions usually were obscured by an abundance of overlying white fat. In cortisone-treated hamsters infected parenterally with the MEF_1 strain, and surviving beyond 5 days, the masses of brown fat were transformed to dense, chalk-white, opaque bodies which were clearly apparent within the surrounding and unaffected white fat.

Site of Brown Fat Necrosis in Relation to Route of Virus Inoculation

When the MEF_1 strain of poliomyelitis virus was inoculated intraperitoneally into a cortisone-treated hamster, the primary microscopic lesions were confined to the brown fat encasing the adrenal glands, and became evident after about 4 days. Within 24 hours thereafter, typical fat necrosis could be demonstrated in the neighboring paravertebral brown fat; and within a further 48 hours, if the animal survived, the interscapular and axillary deposits of this tissue were demonstrably involved. The primary brown fat lesions following intramuscular inoculation of MEF_1 virus into a front limb of a cortisone-treated hamster were located in the ipsilateral axillary brown fat and the interscapular brown fat body. In the subsequent 48 hours, if death did not supervene, steatopathy was seen in the contralateral axillary, periadrenal, and paravertebral loci of brown fat. Subcutaneous inoculation of the virus into an upper limb led to a distributive pattern basically similar to that seen following intramuscular introduction of the virus. Lesions of brown fat also were evident about 5 days after intracerebral injection of the virus, if concurrent cortisone was used. Lesions in such animals tended to be less intensive than in groups infected parenterally, but no specific primary localization was apparent. There was thus a biphasic pattern of brown fat involvement following experi-

mental infection with parenterally introduced poliomyelitis virus. The depots of brown fat nearest the site of viral inoculation showed necrosis within 72 hours. Later in the course of the disseminated disease, as well as in the intracerebrally infected animals, other principal regions of brown fat became involved. The regional correlation which existed between the site of entry and the initial locus of brown fat necrosis was substantiated by daily virologic titrations of the various tissues.[6]

The mode of virus transport, while suggestively vascular, was in doubt. Some investigators have stressed axonal conduction of the virus, *in vivo*, as the sole means of transportation.[10] The applicability of this hypothesis was tested in the light of the findings discussed in this paper. Studies of the nerve distribution to the interscapular brown fat body were carried out. It was determined that the very few, afferent, neural fibers which traversed this structure entered from the ventral plane in the midsagittal line. Total denervation was accomplished on 5 hamsters by resecting the entire interscapular body from the posterior thoracic cage. The dorsal attachment to the interscapular subcutaneous tissue served as the sole source of blood supply. The interscapular brown fat body was returned to its former location and the cutaneous incision closed. After a period of 5 days the animals were inoculated with MEF_1 virus in an upper extremity and given the previously described course of cortisone. Four days following the virus injection the animals were sacrificed. Microscopic examination of the interscapular body showed a moderate degree of non-specific necrosis, attributable to the surgical intervention as well as to the critically reduced vascular supply. In the viable portions of brown fat, however, there was extensive, intracellular necrosis of the type associated with MEF_1 virus propagation. Specific impregnation stains failed to disclose any intact axons. The denervated brown fat was tested also for virus content by intracerebral injection of mice with serially diluted emulsion. Infective levels, considerably above the concentration initially inoculated, and of the same magnitude obtained without nerve resection, were obtained.

The Rôle of Cortisone in Brown Fat Necrosis

No visceral lesions in striated muscle or brown fat were observed in normal hamsters inoculated with MEF_1 virus parenterally. Poliomyeloencephalitis, in the absence of cortisone, was initiated only after direct intracerebral administration of the virus. Hence cortisone therapy was an obligatory background for either visceral or nervous system implication following peripheral inoculation of the virus in the hamster.

The following experiments were carried out in order to determine whether the virus could proliferate and elicit lesions in the brown fat in the absence of cortisone. Thirty male hamsters were used. Vertical incisions were made in the posterior thoracic region exposing the interscapular brown fat body. Twenty animals were inoculated directly into this fatty structure with 0.1 cc. of MEF_1 (dilution, 1:20). Cortisone was administered to 5 of these hamsters. Five additional control animals were inoculated with 0.1 cc. of a normal mouse brain emulsion (dilution, 1:20). A further control group of 5 hamsters received 0.1 cc. of virus (dilution, 1:20), under direct visualization, in the inguinal white fat pad. No cortisone was administered to this latter group.

Animals receiving intramuscular cortisone as well as direct virus inoculation into brown fat showed early paralysis and rapidly succumbed. Microscopic sections of the interscapular body disclosed intensive and plenary damage to the brown fat cells. The resultant degree of necrobiosis was perceptibly greater than in cortisone-treated animals receiving the virus by the usual parenteral routes. Death ensued so rapidly that no lesions were apparent in other brown fat depots of the body.

Animals submitted to the same procedure, with the omission of cortisone, never developed paralysis. Hamsters in this category, sacrificed after the third day, showed, however, numerous scattered foci of necrosis limited to brown fat. Such lesions often were oriented about small blood vessels and were characterized by a degree of inflammatory reaction not achieved in cortisone-prepared hamsters. Calcification of necrotic brown fat cells, an outstanding feature of cortisone-potentiated poliomyelitis in the hamster, was not seen. It was of interest to note that limited lesions also were produced in axillary brown fat (the mesial aspect of which is in direct apposition to the subscapular extension of the interscapular brown fat mass) but not in the more remote periadrenal, paravertebral, or anterior cervical brown fat. Control studies, using normal mouse brain emulsion as the inoculating medium, failed to produce any changes in the injected tissue, other than needle tract hemorrhage. Animals in which MEF_1 virus was introduced directly into inguinal white fat through an open incision, were sacrificed at intervals, but microscopic sections failed to show any lesions.

A few animals receiving no cortisone and inoculated directly with MEF_1 into the interscapular brown fat body were sacrificed after 4 days and the injected tissue removed. This tissue was emulsified, serially diluted, and reinoculated intracerebrally into series of white mice to determine viral titer. The same procedure was carried out using the injected white (inguinal) fat from other hamsters. Thus it was

found that an appreciable amount of virus proliferated in the brown fat in the absence of cortisone while none could be demonstrated in white fat.

The Effect of Poliomyelitis Virus upon Brown Fat in Mice and Monkeys

Strain MEF_1 (0.1 cc., dilution, 1:20) was administered intraperitoneally or intramuscularly to cortisone-treated mice (Table I). A very small percentage of animals, thus treated, developed paralysis. A number of mice were sacrificed each day up to the 15th day following virus inoculation, and selected tissues were prepared histologically. No myositis was seen.[3] Some regional brown fat lesions were noted in a relatively small number of mice sacrificed beyond the 5th day. The lesions invariably were isolated, involving at most 5 to 10 cells, and were surrounded by an inflammatory cell infiltrate. There was little resemblance to the confluent, intensely necrotic and calcified changes exhibited in hamster brown fat. Poliomyelitis virus, in moderate titer, could nevertheless be isolated from mouse brown fat.

Following the demonstration of brown fat in monkeys, the possibility of extraneural proliferation of poliomyelitis virus in such tissue was explored. Cortisone treated and untreated cynomolgus monkeys were inoculated parenterally with various strains of type 1 poliomyelitis virus (Wisconsin, MacMahon, Brabyn). Surgical specimens of brown fat from the axillary region were studied histologically as well as tested for virus content. As previously shown,[9] irrespective of cortisone treatment, 5 days after intramuscular injection of the virus into the upper limb, focal necrotic lesions appeared in the ipsilateral axillary brown fat. Involved cells rapidly underwent degeneration and were obscured by the reactive inflammatory response. No secondary calcification occurred. In contrast to the viral necrosis of brown fat in the hamster, the lesions were isolated by broad, intervening regions of morphologically intact brown fat. The specificity of the simian brown fat lesions was strengthened by the demonstration of high titers of virus in the brown fat displaying necrobiosis. A limited number of cynomolgus monkeys were inoculated parenterally with a type 3 strain of poliomyelitis (Farabaugh). Lesions of brown fat again were observed and virus isolated.

Effect of Related Viruses upon Brown Fat of Hamsters

The lipotropic capacities of two additional strains of type 2 poliomyelitis were investigated. The Lansing strain was injected intraperitoneally (0.5 cc., dilution, 1:50) into 57 hamsters, 37 of which received

supplemental intramuscular cortisone. Approximately 40 per cent of the hamsters in the cortisone treated category showed clinical evidence of paralysis. No paralysis ensued when the hormone was omitted. Steatopathy restricted to the periadrenal and paravertebral brown fat was seen in animals displaying paralysis. Such lesions were qualitatively similar to the MEF_1 virus-induced necrosis of brown fat, but were more limited in distribution and intensity. The Lansing strain is recognized as less virulent than the MEF_1 strain.

The Yale-SK strain of poliomyelitis virus was inoculated intramuscularly (0.2 cc.; dilution, 1:20) into 12 hamsters, 6 of which were given 12 mg. of cortisone in four divided doses. Cortisone produced a significant enhancement of the disease, resulting in an 83 per cent morbidity as compared to a total absence of paralysis or other symptoms in the group not receiving cortisone. Visceral lesions were confined to cortisone-prepared hamsters developing paralysis. In animals sacrificed 3 days after virus administration, a few isolated foci of necrosis were seen in the ipsilateral axillary brown fat. Lesions of a more intense character were found in the ipsilateral axillary and interscapular masses of brown fat after 4 days. In animals surviving 5 days there was widespread confluent necrosis involving all regions of somatic brown fat. The pathogenesis and the ultimate microscopic appearance were identical to those described for the MEF_1 strain. Secondary calcification was prominent. An accompanying myositis, found principally in the limb receiving the virus, was recorded also.

The effect of Columbia-SK virus upon brown fat was studied. This virus, while not generally considered a member of the human poliomyelitis group of viral pathogens, is closely related in many respects.[11] It is capable of instigating a skeletal myositis in appropriate hosts, which is materially identical to that produced by type 2 strains of poliomyelitis in cortisone-treated animals; and results in an encephalomyelitis in rodents essentially indistinguishable from experimental poliomyelitis.[12] This virus was introduced into 60 hamsters (0.1 cc.; dilution, 1:50) via the subcutaneous, intramuscular, or intracerebral routes. Cortisone was administered to 30 of the animals (Table I). A fatal paralytic infection developed in all animals, but was visibly more severe in those treated with cortisone. Microscopically, an extensive skeletal myositis and early encephalomyelitis were apparent, but no indisputable alteration could be appreciated in brown fat. Rare perivascular infiltrates or interstitial collections of lymphocytes were noted, but at no time was intracellular necrosis or calcification seen. The experiment was repeated using a more dilute inoculum (1:10,000)

in an attempt to retard the violently fulminant course, but in spite of the prolongation of disease, no histologic evidence of brown fat necrosis was forthcoming.

Discussion

Cortisone has been shown to potentiate a wide range of experimental infectious diseases of diverse etiology.[13] The enhancing capacity of this adrenal compound is pre-eminently shown in experimental poliomyelitis of the hamster. Not only are morbidity and mortality increased significantly, but the development of paralytic disease can be effected by various parenteral routes. The observation that poliomyelitis in the hamster can be initiated by pathways other than intracerebral led to the assumption that one or several extraneural tissues were harboring the virus during the preparalytic phase. Histologic studies revealed a series of degenerative changes in skeletal muscle and brown fat adjacent to the region of virus injection. A parallel proliferation of the virus was observed in these soft tissues. It was noted that high infective levels of virus were achieved in brown fat and striated muscle days before involvement of the central nervous system.

It has been stated previously[3] that, under appropriate circumstances, most encephalitis viruses present evidence of myotropism. This affinity for muscle may be apparent only in certain hosts, at specified age periods, or under the influence of cortisone. Of the wide viral spectrum capable of exhibiting this binary tropism, only the Coxsackie and poliomyelitis viruses also proliferate in brown fat. The extraneural histologic lesions of Coxsackie and poliomyelitis viruses are indistinguishable.

The pathologic changes within adipose tissue caused by experimental poliomyelitis infection are additionally provocative because of the exquisite selectivity of involvement. White fat is resistant while brown fat is the site of diffuse and extensive necrobiosis. The selective affinity of the poliomyelitis virus is best demonstrated in areas where both fatty tissues are in intimate apposition. In such regions cellular necrosis ceases abruptly at the line of division. With experimental Coxsackie infection of the suckling mouse, mention has been made of generalized necrotizing steatitis of embryonal fat.[14] Other investigators[15] have reported that in suckling mice infected with the Connecticut 5 and other strains of Coxsackie virus, the lesions were found "in the lobules of embryonal fat in the cervical region, axillae, and interscapular fat pad." The essential modification created by poliomyelitic involvement of brown fat is intracellular and consists of fulminant necrosis of the lipid-bearing cytoplasm. During the course of this breakdown, numerous spherical granules are formed similar to the "F"

granules of experimental Coxsackie infection[16] and poliomyelitis myositis in the hamster.[3] It is tempting to theorize that these structures are elementary bodies as was suggested in connection with Coxsackie infection of visceral tissue. No conclusive evidence for such an assertion is presently available. While these bodies are clearly defined, their staining reactions show no distinction from those of the less circumscribed products of brown fat degeneration. Analogous granules in the lesions of myositis of experimental poliomyelitis were traced to extruded spherules of intermyofibrillar lipid undergoing necrosis.

The singular involvement of brown fat by the MEF_1 strain of poliomyelitis virus indicates that salient differences must exist between this tissue and white fat. The individuality of brown fat, according to Rasmussen,[7] was recorded as early as the 17th century. Hypotheses as to its nature and participation in animal physiology were expressed periodically, without sound factual basis for such speculation. Prior to microscopic study, brown fat was believed to be intimately associated with the thymus. Later, a hematopoietic function was ascribed to it. Histologic investigation disproved these conjectures, and on the basis of morphology and embryogenesis brown fat was classified as a variant of ordinary fat. Studies instituted during this century have disclosed a correlation between the cycle of dormancy and the intracellular constituents of brown fat. One investigator was impressed by the resemblance between brown fat and adrenocortical cells.[8] While an active endocrine rôle for this tissue emerged as an attractive thesis, no proof has been forthcoming.

More recent analyses of these forms of adipose tissue have revealed distinctions other than of color, anatomical distribution, or seasonal variation in constitution. Menschik[17] has demonstrated increased amino-oxidase, cytochrome, oxidase, cholesterol, glucolipids, and phospholipids in the brown fat of guinea-pigs as compared to white fat. Significant physiologic differences between the two varieties of fat also have been noted.[18,19] Studies recorded herein indicate elevated phospholipid, ascorbic acid, alkaline phosphatase, and esterase levels in the brown fat of the hamster. The development of viral steatosis in hamster brown fat is dependent upon the previous or concurrent administration of cortisone. This hormone is incapable of initiating pathologic changes within brown adipose tissue, but can preferentially affect the biochemical constitution of this tissue. Cortisone treatment results in a prompt hypertrophy of brown fat in hamsters, mice, and monkeys,[20] reflected microscopically by a measurably increased cell diameter. In contrast, white fat undergoes moderate atrophy. The effects of the hormone are presumably quantitative since virus can

proliferate if inoculated directly into the brown fat of a normal hamster. The enhancing potentialities of cortisone in experimental poliomyelitis can thus be considered as multiphasic. Cortisone inhibits mesodermal reaction at a peripheral site of inoculation, retarding those defensive mechanisms which inhibit spread of pathogens. The virus is then presumably able to disseminate locally, reaching such neighboring tissues as are receptive to its propagation. Cortisone differentially affects those tissues uniquely amenable to virus growth (i.e., brown fat, muscle) in a direction, apparently, which favors local growth. Inflammatory reaction within brown fat is also repressed. Devastating necrosis ensues, unattended by leukocytic response. Only a belated granulomatous reaction materializes coincident with total cellular deterioration. This phenomenon has been noted previously in the myopathic aspects of cortisone-enhanced experimental poliomyelitis.[8] Cortisone also has been shown to have an effect upon the ultimate target tissue (central nervous system) in subduing the visible expressions of tissue defense to viral invasion.[21]

It has been affirmed by others that the poliomyelitis virus is strictly neurocytotropic and that its appearance in peripheral tissues can be explained on the basis of centrifugal conduction through axons.[10] This hypothesis is untenable in the face of four salient observations: The virus is isolated from brown fat (in the preparalytic period) before there is demonstrable invasion of the central nervous system[5,6]; the level of virus recovered from brown fat far exceeds that found in the central nervous system even after paralysis commences; virus can be demonstrated only in selective extraneural tissues, a finding incompatible with a postulation based upon indiscriminate axonal spread of virus; and virus propagation within brown fat takes place after denervation of this tissue. It must be concluded that the poliomyelitis virus has the capacity, under certain conditions, to initiate independent growth in some extraneural tissues, specifically brown fat and striated musculature.

Summary

Various strains of type 2 poliomyelitis virus, parenterally introduced into cortisone-treated Syrian hamsters, are capable of causing selective necrosis of brown fat. The lesions are characterized by a fulminant cellular degeneration, secondary calcification, and terminally, an early granulomatous reaction. No lesions can be demonstrated in white fat.

A selective lipotropism is seen also in experimental poliomyelitis of monkeys, using type 1 or type 3 strains of the virus. A brown fat steatopathy is evident also in parenterally infected white mice, but

never to the consistent degree noted in poliomyelitis infections of hamsters and monkeys.

In all instances viral propagation within brown fat precedes involvement of the central nervous system, suggesting that this tissue acts as a principal locus of extraneural viral multiplication during the preparalytic phase of the experimental disease.

We wish to acknowledge our appreciation to Mrs. M. Pirogow for the histologic preparations and to Mr. H. Fischler for the photography.

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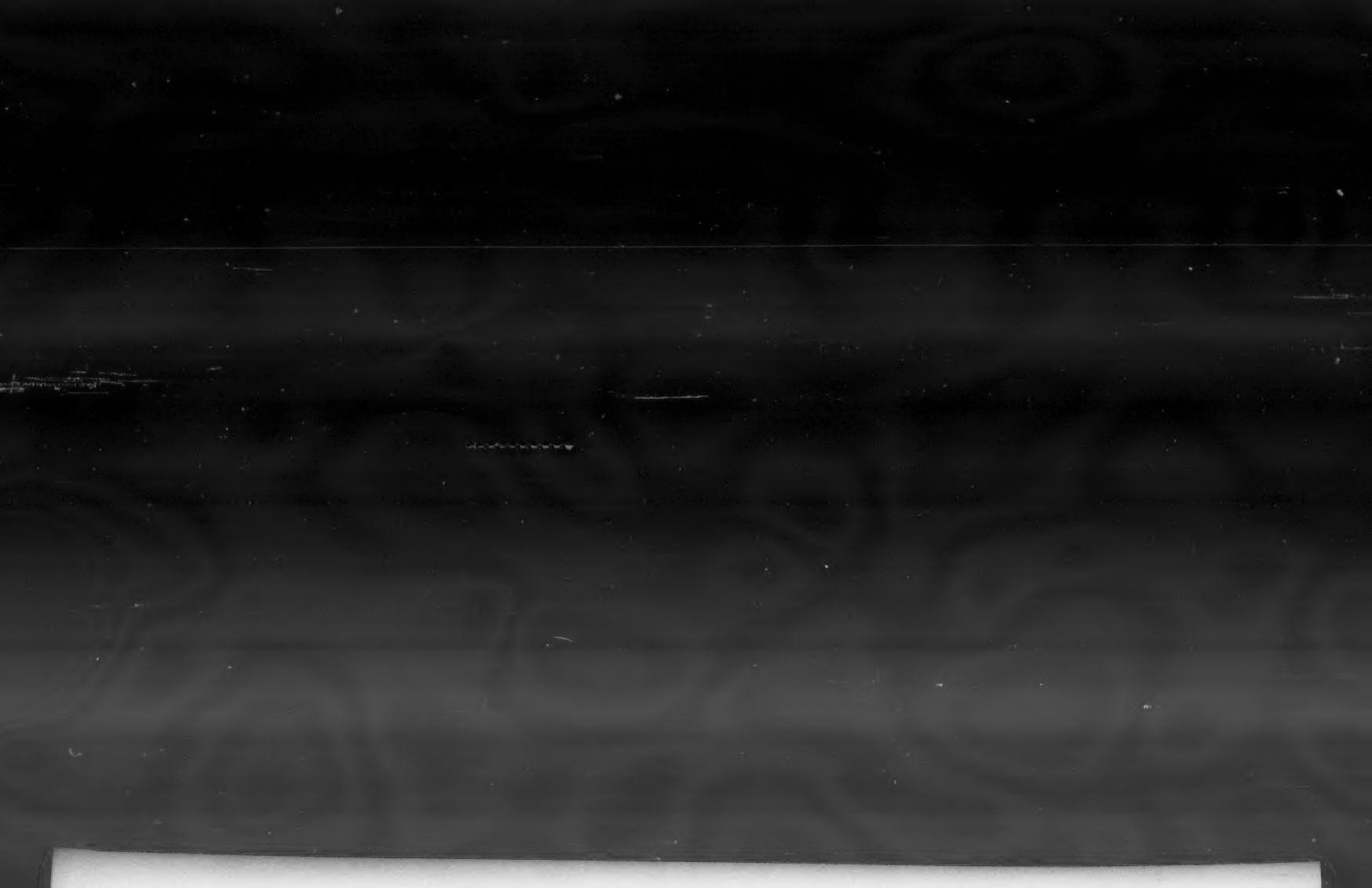
[*Illustrations follow*]

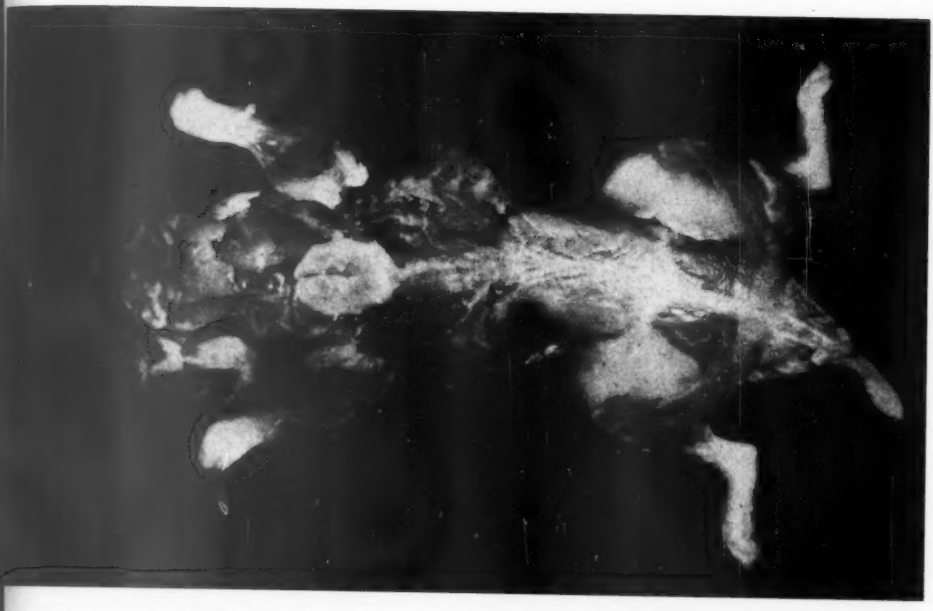
LEGENDS FOR FIGURES

FIG. 1. Syrian hamster with skin dissected away, dorsal view. The midline thoracic, oval-shaped structure is the interscapular hibernating body, composed exclusively of brown fat. Other foci of brown fat are located within the axillary fatty tissues, between the cervical musculature, in the paravertebral and periadrenal regions, and about the thymus.

FIG. 2. Periadrenal brown fat in lower right portion of field. Upper left field is occupied by white adipose tissue. Sharp anatomical delineation between two forms of tissue and difference in respective cell diameters may be noted. Hematoxylin and eosin stain. × 63.

FIG. 3. Brown fat cells, high power, showing centrally located nuclei and delicately structured cytoplasmic network. Hematoxylin and eosin stain. × 453.





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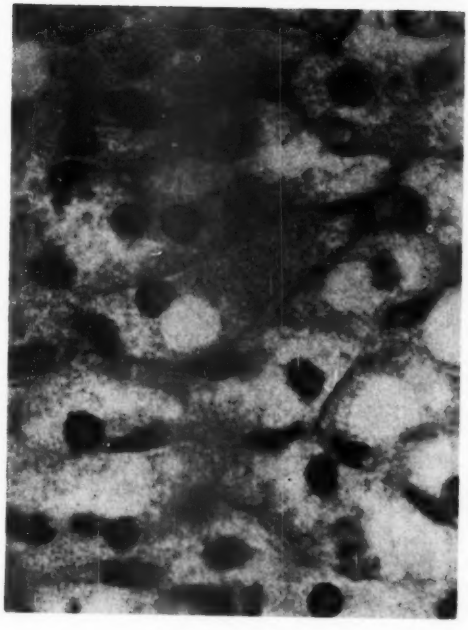

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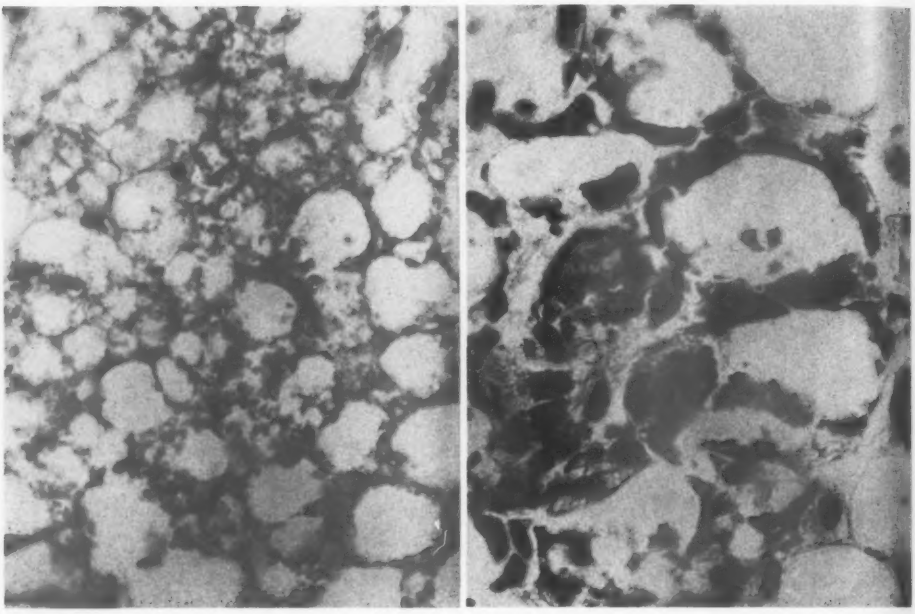


FIG. 4. Brown fat showing early necrosis following parenteral inoculation of MEF_1 virus into cortisone-treated hamster. Intracellular granular necrosis and tendency toward peripheral migration of detritus may be noted. Hematoxylin and eosin stain. × 125.

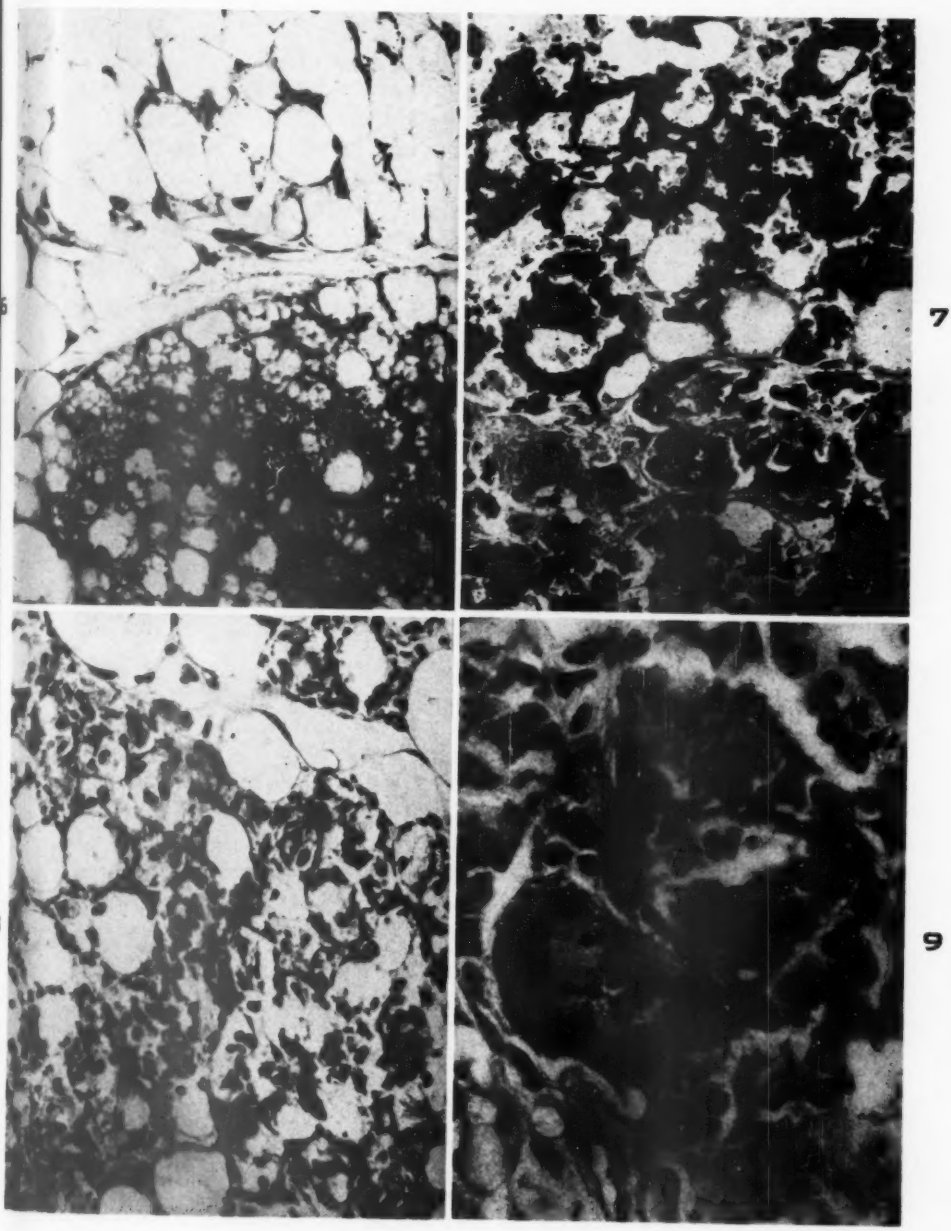
FIG. 5. Brown fat necrosis caused by MEF_1 virus. The degenerating intracellular remnants have coalesced into peripherally situated, coarse, basophilic masses. Hematoxylin and eosin stain. × 453.

FIG. 6. Periadrenal brown fat undergoing viral (MEF_1) necrosis. The histologic changes are confined exclusively to this tissue, the neighboring white fat remaining uninvolved. Hematoxylin and eosin stain. × 90.

FIG. 7. Advanced poliomyelitic viral necrosis of interscapular brown fat. The black staining masses represent areas of reactive calcific deposition. Von Kossa's stain. × 125.

FIG. 8. Advanced viral necrosis of brown fat showing a belated interstitial inflammatory reaction. Hematoxylin and eosin stain. × 90.

FIG. 9. Development of granulomatous reaction with giant cells, in far advanced viral infection of brown fat, occurring 7 days after intramuscular inoculation of MEF_1 virus into a cortisone-treated hamster. × 453.



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THE PATHOLOGY OF TYPE I POLIOMYELITIS IN THE MOUSE *

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Type I poliomyelitis virus recently was adapted to the mouse by Li and Schaeffer,[1] employing the intraspinal route of inoculation. This, together with the earlier adaptation of types II[2] and III,[3] provides an inexpensive virus-host system for investigations of poliomyelitis.

To determine how closely the effects of this poliomyelitis virus in the mouse resemble those in the monkey, the paralytic course and the morphologic changes have been studied. Techniques to facilitate the preparation of the small spinal cords for histopathologic examination are described.

Type I poliomyelitis, originally adapted to 4-weeks-old mice by intraspinal inoculation by Li and Schaeffer,[1] was adapted to mice 3 to 10 months of age by the same route of inoculation. Adaptation to older mice was sought because mature animals have more anatomical uniformity than those at an age of rapid growth.

MATERIALS AND METHODS

Mice. The mice were of strain CFW from colonies maintained at this laboratory. Females 8 to 10 months of age that had been discarded from the breeding colonies were a convenient source of mature animals.

Virus

The source of the virus was the spinal cord of a 4-weeks-old mouse that had become paralyzed several days after intraspinal inoculation of type I poliomyelitis. The history of this strain has been described by Li and Schaeffer.[1] Briefly, it was derived from the Mahoney type I strain, originally isolated from a human case, with subsequent passages in monkeys, tissue culture, and mice (45th passage). This strain has been designated as the LS-a strain.[4]

Intraspinal inoculations were carried out as described by Habel and Li,[5] except that the mid-thoracic region of the spinal cord was chosen instead of the lumbar region as the site of inoculation. By reason of this modification the lumbar and cervical regions, which are of greatest pathologic interest, did not have superimposed lesions of inoculation trauma. Spinal cords were removed for subpassage in the following manner: After death from etherization, the vertebral column was removed from the base of the skull to the lower lumbar region (cauda

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equina of the spinal cord). It was secured in a petri dish with forceps, and sterile saline solution (0.9 per cent) was introduced under pressure into one end of the vertebral canal using a syringe with an 18-gauge needle. Ejecting the spinal cord in this manner is satisfactory for obtaining tissue for passage but is not very satisfactory for preparation of sections for microscopic examination. A simple technique for obtaining good material for microscopic study will be described under histologic techniques.

In the current study, cords were preserved in small glass vials with rubber stoppers secured with adhesive tape. These were stored in the freezing compartment (−10° C.) of a domestic type refrigerator. Spinal cords thus preserved remained infective for at least 5 months. They were prepared for inoculation by grinding with 0.9 ml. of saline solution (0.9 per cent) using a mortar and pestle. The suspension was centrifuged for 5 minutes at 1,500 r.p.m. The supernatant fluid was used for preparation of serial ten-fold dilutions with saline solution. Antibiotics were not added. The routine volume of inoculum was 0.02 ml.

Traumatic paralysis occurs in 10 to 20 per cent of inoculated mice, and is recorded the day after inoculation. Specific paralysis does not appear earlier than the second day after inoculation.

Histopathologic Techniques

The mice were killed with ether at varying intervals after the onset of paralysis. Difficulty was first encountered in attempting to remove small segments of lumbar and cervical spinal cord without damaging them. Further difficulty was experienced in attempting to embed them in paraffin with proper orientation to obtain true cross sections when cut on the microtome. These problems were resolved in the following manner.

For study of the lumbar region, a segment of vertebral column, 3 mm. thick, was removed at or just below the last rib with a scalpel, employing a slicing rather than a crushing motion. The use of scissors was found to be undesirable because their crushing action frequently produced tissue distortion. A similar segment of vertebral column was removed at the level of egress of the nerve plexus to the forelegs. The lumbar and cervical enlargements of the spinal cords of adult mice were found to be at these levels in preparations of sagittal sections of decalcified whole vertebral columns. Vertebral segments containing spinal cord were fixed and decalcified overnight in a solution containing 5 per cent formalin and 5 per cent formic acid. They were washed

briefly in tap water and transferred to 10 per cent neutral formalin[6] for 24 hours or more. They were then dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin. Sections were cut 8 μ in thickness and stained with eosin-methylene blue, citrate-phosphate buffered at pH 5.[6] With the segments of lumbar and cervical spinal cord framed in the decalcified vertebrae, it is easy to embed them in paraffin properly oriented for a true cross section, and manipulative damage to the tissue does not occur. Placement of the vertebrae and segments of spinal cord in buffered neutral formalin after decalcification conditions the tissues for control of the tinctorial properties of buffered eosin-methylene blue stain.

Results

Although this study was primarily concerned with the histopathology and paralytic course of type I poliomyelitis in the mouse, it is of interest to record changes in virus-host relationships which occurred during serial passage in adult mice. For the initial inoculation of adult mice the supernatant fluid from a 10 per cent saline suspension of spinal cord of a paralyzed 4-weeks-old mouse was used. Of 16 adult mice receiving 0.02 ml. intraspinally in the mid-thoracic region, 4 became paralyzed. During successive passages the numbers of mice becoming paralyzed reached 90 to 100 per cent and paralysis and death occurred earlier (second or third day). A 10 per cent cord suspension soon became unsatisfactory for pathologic studies because of death during the night of mice which had not shown paralysis the preceding afternoon. Accordingly, a 10^{-2} dilution was used, subsequently a 10^{-3} dilution, and more recently (17th adult mouse passage) a 10^{-4} dilution was found to be most useful for comparing the paralytic course and the pathologic findings.

In using higher dilutions of cord suspension for successive passages to obtain more desirable material for pathologic study, it is possible that a selection was inadvertently made for more virulent variants. When, by the 10th passage, a few mice became paralyzed following intraspinal inoculation of a 10^{-5} dilution of whole cord suspension, intracerebral inoculation (0.03 ml.) of a 10 per cent suspension was carried out and 2 of 10 mice became paralyzed on the fourth day after inoculation. Intracerebral and intraspinal subinoculation of mice with a 10 per cent cord suspension of one of these mice produced paralysis, but 10 per cent brain suspension failed to do so. With subsequent passages by the intracerebral route this selectivity of higher cord titer has continued. So far (10th passage) in a given passage not more than 30 per cent of mice have become paralyzed. Dilutions of cord suspension

higher than 10^{-1} have failed to produce paralysis. Four-weeks-old mice also are susceptible to this variant by the intracerebral route of inoculation. It has been designated strain LS-b.[4]

Histopathologic Features

The work of Bodian[7] on poliomyelitis in the monkey served as a reference point for this study of the comparative pathology of poliomyelitis in the mouse and monkey. Where there are slight differences in methodology (such as stains employed), these have been controlled by the use of the same methods in current studies being made on poliomyelitis in the monkey.

The normal cyto-architecture of the spinal cords of mice and of monkeys differs in minor respects. The nucleocytoplasmic ratio of the larger motor neurons is smaller in the mouse. While the area of the cytoplasm and, therefore, of the Nissl substance, is less in the mouse, it has the same structure in both species. There is, however, a wider range of variation of depth of basophilia of the Nissl substance in the neurons of the normal mouse. Another difference in the normal neurons of the two species is noted in the staining properties of the nucleoli. When the eosin-methylene blue stain is buffered in a range (pH 5) that is optimum for the demonstration of chromatolysis of the Nissl substance in poliomyelitis, the nucleolus of the normal monkey neuron is deeply basophilic while that of the normal mouse neuron is weakly basophilic. The degree of basophilia of the nucleoli of normal mouse neurons varies somewhat, but in normal cells it is not as intense as it is in poliomyelitis-infected cells.

In the spinal cords of mice necropsied within 1 or 2 hours after the onset of paralysis of a leg, one finds some normal neurons and others in various stages of degeneration (Fig. 2). At this stage of the disease there is no leukocytic infiltration. Only after several days of paralysis does one begin to find diffuse infiltration of leukocytes in the anterior horn regions (Figs. 3 and 4). While this diffuse leukocytic infiltration is consistent in the mouse as well as in the monkey, focal clusters of leukocytes (Fig. 4) occur irregularly in the mouse. Sometimes disappearance of neurons occurs by lytic changes, leaving cavities; or one sees evidence of removal of neuronal débris by leukocytes ("neuronophagia"). The latter is not seen as frequently in the mouse as it is in the monkey.

The progression of changes in the motor neurons is as follows. There is a progressive loss of depth of basophilia of the cytoplasm. The normally sharp outlines of the Nissl substance (Fig. 5) are lost and the cytoplasm has a ground-glass appearance (Fig. 6). Lytic changes then

set in (Fig. 7), leaving a cavity, or the neuronal débris is infiltrated with leukocytes ("neuronophagia") (Fig. 8).

Perivascular cuffs of leukocytes are found in the spinal cord only after neuronal damage and accompanying paralysis have set in. They do not appear uniformly throughout the spinal cord. For example, after hind leg paralysis of several days' duration, one finds loss of large motor neurons with perivascular cuffs in the lumbar region but not in the cervical region even though early paralysis and neuronal damage have set in.

While the changes in the neurons leading to irreversible damage are very similar in the mouse and the monkey, recovery of some neurons, as occurs in the monkey, has not been observed in the mouse. When a dilution of cord material is used, which, when inoculated intraspinally, produces paralysis in about half of the mice, symptoms appear on the 3rd to 6th day after inoculation. The spinal cords of mice which failed to become paralyzed by the 14th to 21st day were examined in considerable numbers. In no instance was there found a decrease in population of motor neurons, evidence of neuronal damage with recovery, or leukocytic infiltration (findings characteristic of mild paralytic poliomyelitis with recovery in the monkey[7]).

Another slight dissimilarity of pathologic reaction in the two species is the relative frequency of neuronophagia. Slight diffuse leukocytic infiltration occurs consistently in both species. Clusters of leukocytes at former sites of neurons, however, are not seen consistently in the mouse. When leukocytes are not present, cavities are found at such sites.

Some singular aspects of poliomyelitis in primates are the localized sites of neuronal damage, the finding of different stages of damage in different neurons at the same time, and the fact that neuronal damage clearly precedes focal and perivascular infiltration of leukocytes. These pathognomonic features of poliomyelitis in primates are also found in the mouse.

A correlation exists between the location and duration of paralysis of one or more legs and the location and stage of neuronal degeneration in the spinal cord. When a single hind leg is paralyzed, extensive neuronal damage is found in one anterior horn of the lumbar cord but not in the other. When there has been hind leg paralysis of several days' duration and the animal is sacrificed soon after the forelegs also have become paralyzed, all motor neurons are destroyed in the lumbar region and very early neuronal damage is seen in the cervical region of the spinal cord.

Cerebral lesions have not been found in mice that have been sacri-

ficed or were found dead after the appearance of paralysis following intraspinal inoculation. On the other hand, perivascular cuffs of leukocytes have been found in brains of some mice that have become paralyzed following intracerebral inoculation of 10 per cent suspensions of spinal cords of paralyzed mice (Fig. 9). At present (10th intracerebral cord passage), only 1 to 3 of 10 mice inoculated intracerebrally become paralyzed, so that significant numbers of mice with cerebral lesions have not been available for study. Two important findings are worthy of mention. Chromatolysis and pyknosis occur in the neurons of nuclei in the medulla; and lesions of the spinal cord are more advanced and are seen earlier than cerebral lesions following intracerebral inoculation of spinal cord material from paralyzed mice.

Discussion

Although the initial cost and the cost of maintenance of monkeys are approximately 100-fold that of mice, type I poliomyelitis, which is most commonly found in the human, had not been adapted to mice until recently, thus curtailing the use of the mouse in place of the monkey for some studies. Also, the mouse has not been used appreciably for morphologic studies, presumably because of the difficulties involved in attempting to employ the same techniques as are used in the monkey for the removal of the spinal cord and the preparation of the small specimens for microscopic examination.[8] These technical difficulties have been resolved to the degree that segments of spinal cord of the mouse can be removed and prepared for microscopic examination more easily than can be done in the monkey.

The similarity of changes in the spinal cord produced in mice and primates by type I poliomyelitis confirms the finding of such a similarity by Lillie and Armstrong,[9] Jungeblut and Sanders,[10] and Ehrich and Foster[11] for type II virus. In the studies of Ehrich and Foster, the intracerebral route of inoculation was employed and cerebral lesions were found in some but not all paralyzed mice. In the current study, cerebral lesions were not found in mice that had become paralyzed following intraspinal inoculation. The reason for this is not clear but it is probably due to the special affinity of this virus strain for anterior horn neurons since it was "adapted" by Li and Schaeffer[4] as a "spinal variant." As evidenced by paralysis and microscopic findings, virus clearly migrated from the site of inoculation in the mid-thoracic region to the cervical and lumbar regions of the spinal cord. However, there was not sufficient migration of the virus to produce cerebral lesions such as were found following intracerebral inoculation. Moreover, death of the infected mice cannot be attributed necessarily to cerebral invasion

of the virus since paralyzed mice lose weight rapidly and death is probably due to inanition.

Perivascular cuffs of leukocytes and pyknotic neurons were seen in the brains of some but not in all mice that had typical changes of the spinal cord following intracerebral inoculation. A similar finding was reported by Lillie and Armstrong.[9]

The cerebral perivascular cuffs appear after neuronal damage occurs in the spinal cord. Some sections have been made of the brain at the site of inoculation. In these one finds a glial scar with only sparse leukocytes as compared with thick perivascular cuffs of leukocytes.

One of the interesting aspects of cell-virus-relationships of poliomyelitis in primates is that many damaged neurons recover.[7] Such a phenomenon has been sought for meticulously in the present study and no evidence for its occurrence has been found. Mice rarely survive more than 2 or 3 days after the onset of paralysis. Dehydration from inability to reach the drinking tube is probably a large contributing factor in early deaths. The day before onset of paralysis mice frequently exhibit sluggish behavior but normal muscular coordination. At times they will continue thus for 2 or 3 days and then become active and vigorous in their movements without showing any paralysis. Spinal cords of such mice have been examined and there have been found no abnormal neurons, no decrease of motor neurons, nor any leukocytic infiltration that would be consistent with non-paralytic poliomyelitis or with transient paralytic disease with recovery. In primates that have recovered from mild, transient, paralytic poliomyelitis, typical changes are seen in neurons immediately after such recovery and focal and perivascular infiltration of leukocytes persists for months.[7]

Hyden[12] reviewed the changes of nucleic acid in neurons in poliomyelitis in the monkey and in man. Ultraviolet microspectrographic and other cytochemical techniques were employed. The earliest change in the poliomyelitis-infected neuron occurs in the nucleolus, there being a rapid increase in ribose nucleotide content. There is concurrent increase of ribose nucleic acid in the cytoplasm. In the next phase the nucleolus shows signs of damage, with loss of nucleoproteins, and nucleotides of the cytoplasm disappear. In the current study, changes in basophilia of components of the neurons appeared to coincide with changes in amount of ribose nucleic acid. In sections stained with buffered eosin-methylene blue, the first change consisted of the appearance of deeper basophilia of the nucleolus than was seen in normal neurons. This was followed by decrease of basophilia of the cytoplasm, and later by pyknosis. This apparent correlation between the amount of ribose nucleic acid and the degree of basophilia is not to be con-

strued as a generalization. It appears, however, to be a rational interpretation in the case of the mouse neuron in poliomyelitis.

Although spinal cord and brain were not titered for virus content in the current study, evidence of higher spinal cord titer was obtained by infectivity of cord-to-brain passage and lack of infectivity of brain-to-brain passage using a 10 per cent suspension. Stanley *et al.*[13] and Krech[14] also adapted the Li and Schaeffer strain LS-a to mice employing the intracerebral route of inoculation. Both also found evidence of higher titer of virus in the cord than in the brain.

In their studies of type II poliomyelitis in the mouse, Ehrich and Foster[11] also found a correlation between the sites of neuronal damage and the locations of paralysis, as well as between the stages of pathologic changes and the duration of clinical symptoms. This, together with the similarity of nuclear and cytoplasmic changes in the anterior motor neurons to those found in primates, serves to re-emphasize the statement of these authors: "From the foregoing observations it is concluded that the mouse is well suited for the experimental study of poliomyelitis, particularly when large numbers of animals are required."

Summary

The histopathologic features of type I poliomyelitis in the mouse has been compared with those in primates and found to be similar in general. Some slight differences in the normal cyto-architecture of the neurons and their reaction to the virus in the two species are described. Simplified techniques have been developed for preparation of the spinal cords of mice for microscopic examination. There seems little doubt of the efficacy of this less expensive host for certain types of poliomyelitis research.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All photomicrographs are from sections stained with buffered eosin-methylene blue.

FIG. 1. Anterior horn region of the spinal cord of a normal mouse. × 200.

FIG. 2. Anterior horn region of the spinal cord of a mouse shortly after onset of paralysis following intracerebral inoculation with type I poliomyelitis virus. There is a single normal neuron with deeply basophilic cytoplasm (upper right). Other neurons show stages of chromatolysis. × 200.

FIG. 3. Early stage of diffuse and focal leukocytic infiltration of the anterior horn region of the spinal cord. × 200.

FIG. 4. Later stage of leukocytic infiltration. The clusters of leukocytes represent areas of neuronophagia. × 200.

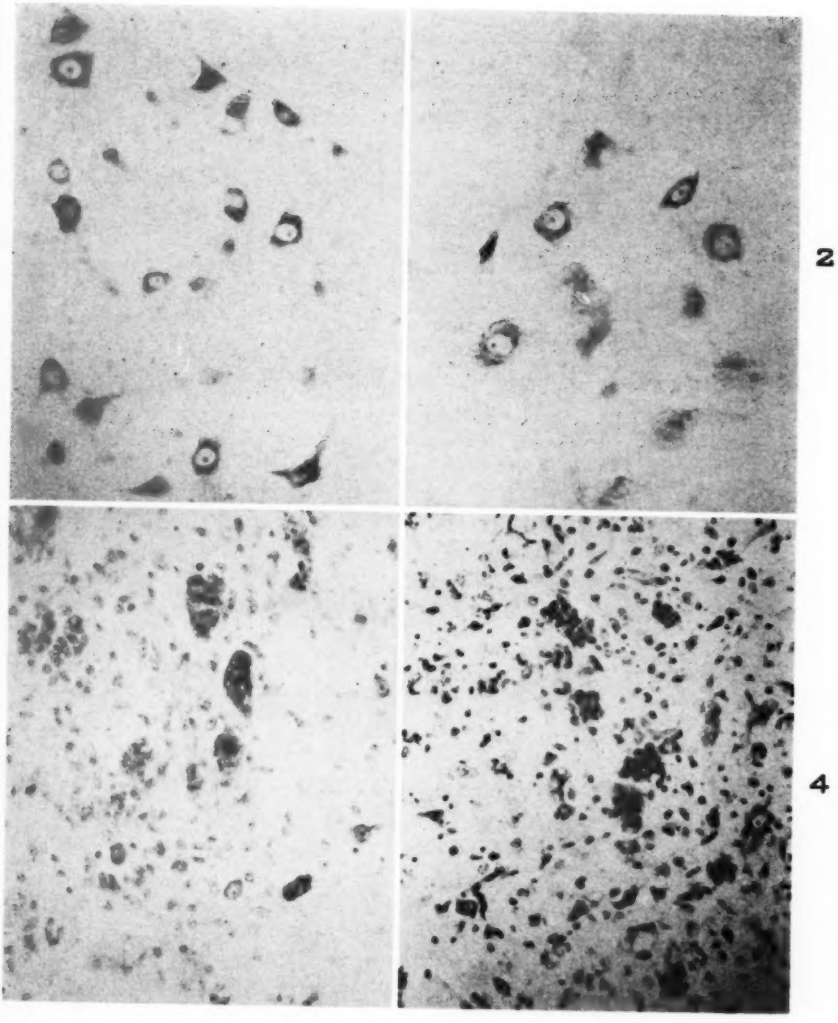
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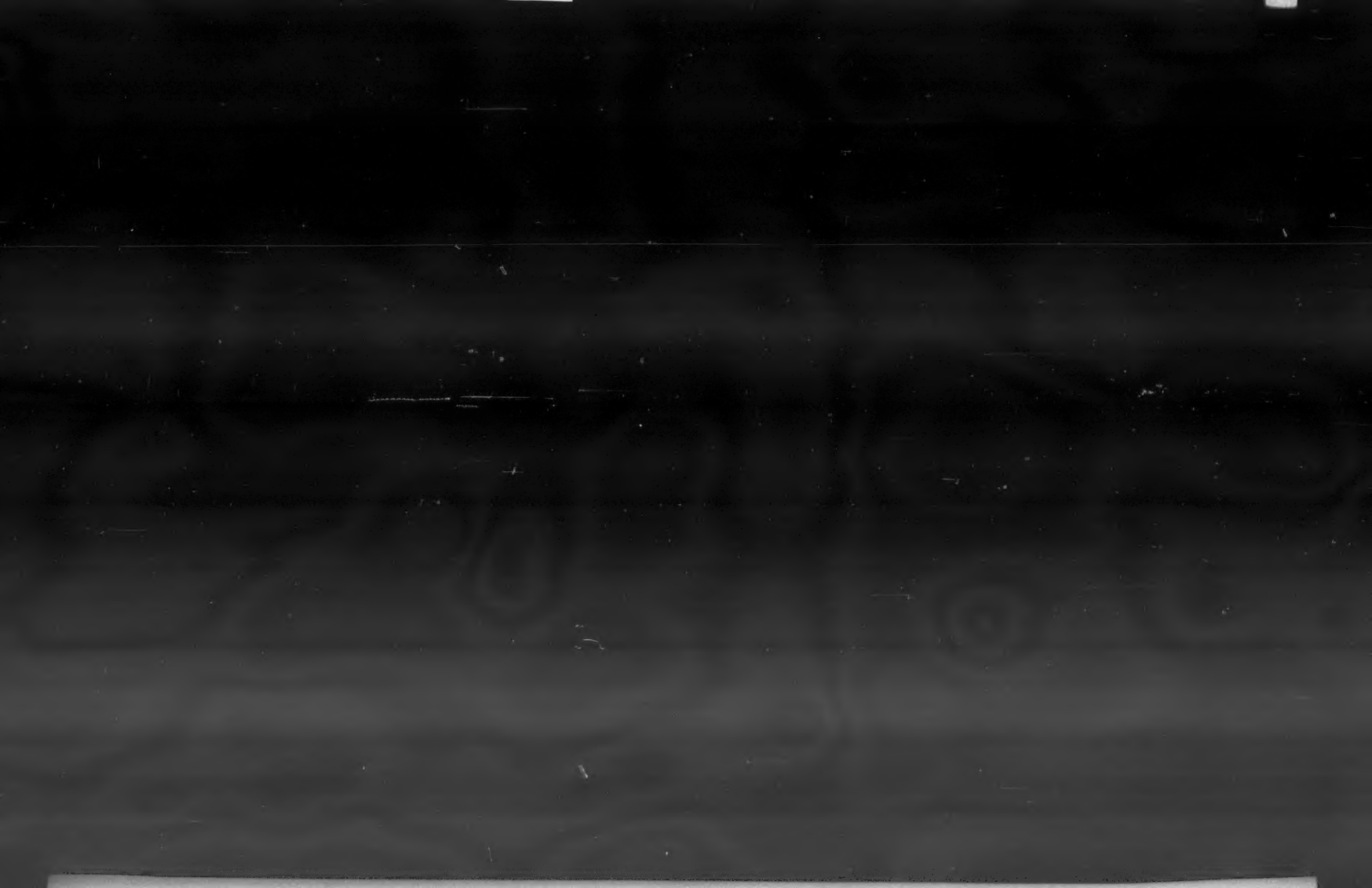
FIG. 5. Normal motor neuron in the anterior horn region of the spinal cord. × 1,000.

FIG. 6. Chromatolysis of motor neuron characteristic of poliomyelitis. × 1,000.

FIG. 7. Late stage of neuronal damage in poliomyelitis. × 1,000.

FIG. 8. Cluster of leukocytes around a damaged neuron, "neuronophagia." × 1,000.

FIG. 9. Perivascular leukocytic infiltration in the brain of a mouse showing typical changes in the spinal cord, following intracerebral inoculation of a suspension of spinal cord from a paralyzed mouse. × 200.



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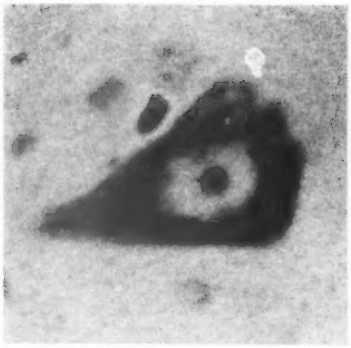

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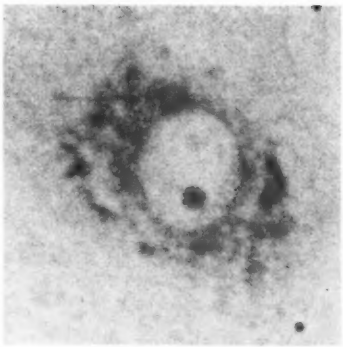

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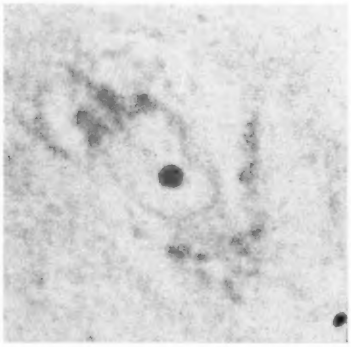

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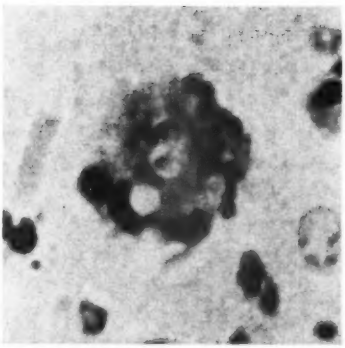

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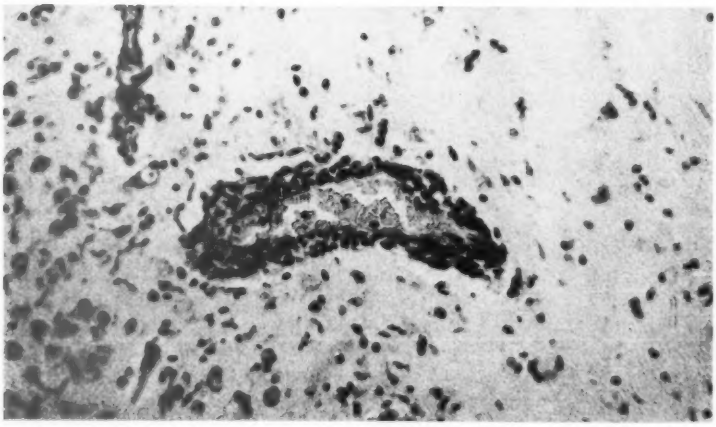

FACTORS INFLUENCING THE STAINING OF BETA-CELL GRANULES IN PANCREATIC ISLETS WITH VARIOUS BASIC DYES, INCLUDING PARALDEHYDE-FUCHSIN *

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Gomori,[1] in 1950, reported that basic fuchsin, in the presence of strong mineral acids, forms an intensely purplish dye with certain aldehydes. The dye was referred to as *aldehyde-fuchsin* and was found to impart a deep purple color to elastic fibers and to a few other tissue structures, among which are the beta-cell granules in pancreatic islets. Gomori[2] considered the staining properties of paraldehyde-fuchsin as being unique in the sense that no other dye is known to stain both intensely and selectively the beta-cell granules without the influence of prior oxidation.

The effect of prior oxidation upon the paraldehyde-fuchsin staining of various tissue structures has been described by Scott and Clayton[3] and by Scott.[4] They observed that previous oxidation of sections in acidified permanganate for 2 minutes resulted in more rapid and intense staining of pancreatic beta-cell granules than if the sections were treated with Lugol's solution for 30 minutes, as recommended by Gomori.[1] The effect of oxidation with periodic acid was intermediate.

In the present investigation it was found that pancreatic beta-cell granules, after oxidation or bromination, could be stained rapidly and selectively not only with paraldehyde-fuchsin but also with other basic dyes. This article deals principally with such staining reactions, with particular reference to factors that influence them. The staining methods also were applied to purified insulin crystals to determine if any similarity exists between the staining of beta-cell granules and of purified insulin. The principal objective of these studies is to shed some light upon the complex subject of the mechanism of staining pancreatic beta-cell granules with basic dyes.

STAINING OF PANCREATIC BETA-CELL GRANULES WITH BASIC DYES

Material. The material consisted of pancreases obtained from 3 pancreatectomized dogs, from 2 necropsies upon non-diabetic adults, and from one surgical specimen of an insulin-producing islet-cell adenoma. The fixations employed included (1) Bouin's picroformalin

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acetic acid solution (pH, 1.43), (2) formol sublimate buffered with sodium acetate (pH, 5.8), and (3) 10 per cent formalin buffered with calcium acetate (pH, 6.93). Thin (2 to 3 mm.) blocks of pancreatic tissue were fixed for 20 hours. The material was embedded in paraffin and sections cut serially at 5 to 6 μ. Prior to staining, the slides were carried through xylene and graded alcohols to water. In the case of the material fixed in formol sublimate, the mercurial precipitates were removed with Lugol's solution (2 minutes), the iodine then being removed by a rinse in 5 per cent aqueous sodium thiosulfate.

Stock Dye Solutions. The dye solutions employed possessed the following features common to all: (1) constant concentration of the dye in solution (0.50 per cent), except for spirit blue (0.25 per cent); (2) constant use of 60 per cent ethanol for the dye solvent; and (3) preparation and use of the dye solutions at room temperature (about 25° C.) and in closed Coplin jars.

For all experiments the following basic triphenylmethane dyes, excepting acid fuchsin which is an acid dye, were employed:

1. Basic fuchsin (C. I. no. 677)
2. Pararosaniline (C. I. no. 676)
3. Crystal violet (C. I. no. 681)
4. Acid fuchsin (C. I. no. 692)

   In selective instances the following basic dyes were used:

5. Methyl violet 2-B (C. I. no. 680)
6. Thionine (C. I. no. 920)
7. Malachite green (C. I. no. 657)
8. Methyl green (C. I. no. 684)
9. Spirit blue (C. I. no. 689)
10. Standard "cold Schiff" reagent, manufactured according to Lillie's specifications.[5]

Direct Staining with Stock Dye Solutions

With basic fuchsin (pH, 6.9) or pararosaniline, the islet-cell granules were stained pink to red with no differentiation between the alpha- and beta-cell types. With crystal violet (pH, 5.6) or methyl violet 2-B, the islet-cell granules, in general, were colored light to moderate purple with no distinct separation into the granular types. Acid fuchsin (pH, 3) stained the beta-cell granules pink, the alpha-cell granules possessing a more intense shade. Thionine (pH, 3.9) stained the islet-cell granules faint blue with no differentiation between the granular types. In all cases the cytoplasm of the acinar cells was stained similarly to, or more intensely than, the islet-cell granules. Nuclei were

colored orthochromatically, being dull and indistinct only with the Bouin's fixed material. Elastic fibers could not be distinguished readily from the general background staining of connective tissue.

The Effect of Prior Oxidation upon Staining with Stock Dye Solutions. The various oxidizing agents and other pretreatments employed are listed as follows:

1. Periodic acid (H_5Io_6), 1% aqueous solution
2. Potassium permanganate ($KMnO_4$), 0.5% aqueous solution, with 0.5% H_2SO_4 (freshly prepared)
3. Performic acid (HCO_3H), prepared according to Greenspan's[6] specifications; aged 1 hour before use; stable for 1 day
4. Peracetic acid (CH_3CO_3H), prepared according to Greenspan's[6] specifications; aged overnight before use; stable for 2 to 3 weeks
5. Acetic acid control: same concentration of acid as specified for the preparation of peracetic acid; distilled water used in place of hydrogen peroxide
6. Bromine:carbon tetrachloride (Br_2:CCl_4), 1:39 volume dilution.

Deparaffinized sections of pancreatic tissue were treated with one of the above reagents at 25° C. for specified intervals. Following the use of any of the first five reagents, the sections were washed in running tap water for 5 minutes. The sections oxidized with permanganate were treated with 1 per cent oxalic acid to remove the manganese deposits. Following the use of bromine:carbon tetrachloride, the sections were rinsed in 3 changes of carbon tetrachloride and then taken through graded alcohols to water. The sections were stained separately in each of the stock dye solutions for 5 to 20 seconds unless otherwise stated. Following this, they were rinsed quickly in water, mounted in water, and examined immediately.

The results are presented in Table I.

The Effect of pH upon Staining. The stock dye solutions employed in this experiment consisted of basic fuchsin (pH, 6.9), crystal violet (pH, 5.6), and acid fuchsin (pH, 3.0). The pH of each of these solutions was adjusted by adding drops of concentrated HCl. One set of dye solutions possessed a pH of 1.3, and another set a pH between 2.13 and 2.46. Staining was performed both directly and after oxidizaing the sections with peracetic acid for 2 to 3 minutes. After staining, the sections were rinsed quickly with running tap water, mounted in water, and examined immediately.

The results are presented in Table II.

The Effect of Paraldehyde-Dye Mixtures upon Direct Staining. The stock dye solutions employed in this experiment consisted of basic fuchsin, crystal violet, thionine, and acid fuchsin. Three sets of these dye solutions were prepared: one set in which the pH was not adjusted; another set with pH adjusted to 1.3 (this corresponded to 1 per cent HCl in the basic fuchsin solution); and a third set with pH adjusted to 2.1 to 2.5. To 50 ml. aliquots of each dye solution from

TABLE I

The Effect of Prior Oxidation upon Staining Pancreatic Islet-Cell Granules with Stock Dye Solutions

Oxidant or pretreatment	Oxidation interval	Stain (stock dye solution)	Staining interval	Coloration of beta-cell granules*	Coloration of alpha-cell granules*
	2 to 3 min.	Crystal violet or methyl violet 2B	5 to 10 sec.	Brilliant purple-red	Murky blue
	1, 2, 6, and 24 hrs.	Crystal violet	5 to 10 sec.	Dark purple	Murky blue
Peracetic acid or performic acid	2 to 3 min.	Basic fuchsin	5 to 10 sec.	Moderate to dark red	Moderate to dark red
	2 to 3 min.	Malachite green or methyl green	1 to 5 min.	Dull green	Pale green
	2 to 3 min.	Spirit blue	1 to 5 min.	Dull blue	Pale blue
	2 to 3 min.	Thionine or acid fuchsin	1 to 30 min.	Faint color or colorless	Faint color or colorless
	2 to 3 min.	Schiff's reagent	15 min.	Colorless	Colorless
Potassium permanganate, acidified (0.5% aqueous)	5 to 10 min.	Crystal violet or methyl violet 2B	5 to 15 sec.	Brilliant purple-red	Dull blue
	5 to 10 min.	Basic fuchsin	10 to 15 sec.	Dark red	Dark red
	5 to 10 min.	Schiff's reagent	15 min.	Colorless	Colorless
Periodic acid (1% aqueous)	2 hrs.	Crystal violet	5 to 10 sec.	Dark pinkish purple	Pale purple
	6 and 24 hrs.	Crystal violet	5 to 10 sec.	Brilliant purple-red	Murky blue
	30 min.	Schiff's reagent	15 min.	Colorless	Colorless
Bromine:carbon tetrachloride (1:39 volume dilution)	15 to 60 min.	Crystal violet or methyl violet 2B	5 to 10 sec.	Dark purple	Pale purple
	45 min.	Schiff's reagent	15 min.	Colorless	Colorless
Acetic acid control	2, 10 min.; 1, 6, and 24 hrs.	Crystal violet	5 to 15 sec.	Colorless	Colorless
No pretreatment		Crystal violet, methyl violet 2B, or basic fuchsin	5 to 15 sec.	Colorless	Colorless

* Sections mounted in water and examined immediately.

each set there was added 0.5 ml. of paraldehyde (aldehyde-free). Thus, the basic fuchsin solution containing 1 per cent HCl and 1 per cent paraldehyde corresponded to Gomori's specifications for the preparation of paraldehyde-fuchsin. The solutions were allowed to age at 25° C. for 15 hours and for 48 hours before use. The added paraldehyde did not influence significantly the pH of the solutions. Deparaffinized sections of pancreatic tissue were immersed separately in each dye

TABLE II

The Effect of pH upon Staining Pancreatic Islet-Cell Granules

Stain	pH of dye solution	Staining interval	Oxidation prior to staining	Coloration of beta-cell granules*	Coloration of alpha-cell granules*
Basic fuchsin	1.3	6, 10, and 30 min.	None	Pale pink	Pale pink
	2.3	6, 10, and 30 min.	None	Pink	Pink
	6.9	1 to 5 min.	None	Pink to red	Pink to red
	1.3 and 2.4	3 to 10 min.	Peracetic acid (2 to 3 min.)	Bright red	Pale pink
	6.9	5 to 10 sec.	Peracetic acid (2 to 3 min.)	Moderate to dark red	Moderate to dark red
Crystal violet	1.3	6, 10, and 30 min.	None	Faint purple	Faint purple
	2.3	6, 10, and 30 min.	None	Pale purple	Pale purple
	5.6	1 to 5 min.	None	Light to moderate purple	Light to moderate purple
	1.3 and 2.3	1 to 2 min.	Peracetic acid (2 to 3 min.)	Dark purple	Faint purple
	5.6	5 to 10 sec.	Peracetic acid (2 to 3 min.)	Brilliant purple-red	Murky blue
Acid fuchsin	1.3, 2.2, and 3.0	5 to 6 min.	None	Pink	Pale red
	1.3	5 to 10 sec.	Peracetic acid (2 to 3 min.)	Pink	Red
	3.0	1 min.	Peracetic acid	Faint pink	Faint pink

* Sections mounted in water and examined immediately.

solution for 1 to 10 minutes. The sections were examined through a water mount as before.

The results are summarized as follows: Direct staining with the various paraldehyde-dye mixtures, excepting paraldehyde-basic fuchsin, led to results similar to those with direct staining in the same dye solution of corresponding pH but without added paraldehyde. Direct staining with paraldehyde-basic fuchsin at pH 1.3 or 2.3 and

aged for 15 hours resulted in bluish red beta granules and faint pink alpha granules. Elastic fibers were colored a deep bluish red. After the dye solution had aged 48 hours, the beta granules and elastic fibers were stained a deep purple or purple-red. The paraldehyde basic fuchsin solution at pH 6.9 did not give rise to differential islet-cell staining.

The Effect of Prior Oxidation upon Staining with Paraldehyde-Fuchsin. The reagents and the procedures employed in this experiment were identical with those described under the heading "The effect of prior oxidation upon staining with stock dye solutions." The only difference in the present experiment was that staining was performed with Gomori's paraldehyde-fuchsin solution, aged 48 hours before use. The staining interval was 10 to 15 seconds. Non-oxidized controls were carried along for each fixative.

The results are presented in Table III.

Destaining Procedures. The stained sections in the experiments just described were examined microscopically through a water mount.

TABLE III

The Effect of Prior Oxidation upon Staining Pancreatic Beta-Cell Granules with Paraldehyde-Fuchsin, Aged 48 Hours

Oxidant or pretreatment	Oxidation or pretreatment interval	Coloration of beta-cell granules*
Peracetic acid	2 to 3 min.	Deep purple
	2, 6, and 24 hrs.	Deep purple
Performic acid	2 to 3 min.	Deep purple
Acetic and formic acid controls	2 to 10 min.; 1, 6, and 24 hrs.	Colorless
Potassium permanganate, acidified (0.5% aqueous)	5 to 10 min.	Deep purple
Periodic acid (1% aqueous)	2 to 15 min.	Pale purple
	1.5 hrs.	Purple
	2 hrs.	Deep purple
	24 hrs.	Deep purple
Bromine:carbon tetrachloride (1:39 vol. dil.)	5 to 10 min.	Light purple
	30 min.	Deep purple
No pretreatment		Faintly colored or colorless

* Staining interval: 10 to 15 seconds. Direct staining for 3 to 10 minutes, depending upon the type of fixation used, was necessary to give results equivalent to those with staining for 10 to 15 seconds after oxidation or bromination.

These sections then were treated with a variety of organic solutions, listed as follows:

1. 70% ethanol containing 1% HCl, referred to as "acid-alcohol" (pH, 1.34)
2. 1% aqueous solution of borax ($Na_2B_4O_7 \cdot 10H_2O$), referred to as "borax solution" (pH, 9.16)
3. Sequence of 95% ethanol, 100% ethanol, ethanol-xylene (equal parts), xylene, and permount
4. Sequence of acetone, acetone-xylene (equal parts), xylene, and permount

The *results* are summarized as follows: The sections of pancreas stained by any of the basic dye solutions with or without prior oxidation were decolorized completely by acid-alcohol within 2 minutes but largely resisted decolorization by the borax solution for 30 minutes. This applied to all the pancreatic structures, including beta-cell granules, that were stained by a given method. Decolorization with acid-alcohol was extremely rapid (few seconds) in beta granules stained by crystal violet and least rapid (1 to 2 minutes) in those stained by paraldehyde-fuchsin. The pancreatic structures, including the alpha-cell granules, that were stained by acid fuchsin were decolorized by the borax solution within 2 minutes but largely resisted decolorization by acid-alcohol for 30 minutes. The coloration of beta granules by crystal violet was extracted completely in 95 to 100 per cent ethanol within 1 minute and in acetone within 3 minutes. The metachromatic staining of beta granules by crystal violet after peracetic acid oxidation was transformed into an orthochromatic coloration when the sections were dehydrated rapidly in the acetone sequence. By this rapid dehydration method the beta granule staining was extracted minimally, whereas acinar-cell staining was decolorized to a variable extent within the same section. The usual non-rapid dehydration sequence in ethanol resulted in a minimal loss of dye from sections stained with paraldehyde-fuchsin. Actually, the intensity of beta granule staining did not appear to be affected. Beta granules stained by crystal violet or by paraldehyde-fuchsin not only could be decolorized completely with acid-alcohol but also could be restained in undiminished intensity. In other words, under the conditions employed, the use of decolorizing agents did not result in dissolution of the beta granules.

*The Effects of Various Fixations upon Direct Staining with Paraldehyde-Fuchsin.** The effects of varied fixations upon direct staining of

* Part of the experimental data reported herein was obtained through the courtesy of Dr. G. L. Laqueur, Miss E. Lee, and Mrs. E. Siperstein at the National Institutes of Health, Bethesda 14, Md.

beta-cell granules with paraldehyde-fuchsin are summarized as follows: With tissue fixed in buffered formol sublimate, the optimal interval for staining beta granules varied between 1.5 and 3 minutes. With tissue fixed in Bouin's fluid or in 10 per cent formalin, the optimal interval was 10 minutes. With staining for intervals longer than these, there was no appreciable change in the intensity of beta granule coloration, though the general background staining was accentuated. Under these conditions of staining, the beta granules were colored brilliantly in tissue fixed in formol sublimate or in Bouin's fluid, and slightly less brilliantly in tissue fixed in 10 per cent formalin. Under these conditions of staining, the general background coloration was nil with tissue fixed in Bouin's fluid or in 10 per cent formalin, and was slight to moderate in tissue fixed in formol sublimate. The short (1 to 2 minute) treatment of sections with Lugol's solution, necessary to remove mercury precipitates from tissue fixed in formol sublimate, did not affect the results of staining when applied to tissues fixed in Bouin's fluid or in 10 per cent formalin. Thus, under the conditions employed, the use of Lugol's solution does not appear to be a factor in accelerating the staining of beta granules in tissue fixed in formol sublimate. Fixation of tissue in absolute alcohol or in Zenker's fluid was found to be unsuitable.

Factors That Affect the Staining Properties of Paraldehyde-Fuchsin Dye. The following factors influenced the staining properties of the paraldehyde-fuchsin dye: (1) The use of paraldehyde solutions from different sources seemed to alter somewhat the ability of paraldehyde-fuchsin to stain the beta granules. The paraldehyde which had been most satisfactory in our hands was a preparation sold at a local pharmacy for medicinal purposes. (2) The use of commercial basic fuchsin dyes of low maximal spectral absorption (435 to 440 mμ) failed to produce paraldehyde-fuchsin dyes which were satisfactory for staining beta granules, though grossly a purple dye was formed. This confirms the observations of Gomori.[2] (3) Paraldehyde-fuchsin dyes which had aged over a prolonged period (e.g., 2 months at 25° C.) failed to stain the beta granules directly. However, after selective oxidation, the granules could be colored about as quickly and intensely as with dye preparations aged for short intervals.

Summary of the Results of Staining Pancreatic Beta-Cell Granules with Basic Dyes

Basic triphenylmethane dyes, such as basic fuchsin, methyl violet 2-B, or crystal violet, were capable, in the absence of HCl and of paraldehyde, of staining elastic fibers and beta-cell granules in

pancreatic islets. However, this direct staining resulted in such a generalized and intense coloration of pancreatic structures that differentiation between the connective tissue fibers or the granular types in the islets was difficult or impossible. Lowering the pH of these dye solutions led to a depression of their affinity for various pancreatic structures, but the islet-cell granules, though stained less intensely, still could not be distinguished readily as two types. Only paraldehyde-fuchsin was capable of staining, both directly and selectively, the beta-cell granules.

Oxidation of sections in performic or peracetic acid for 2 to 3 minutes had a pronounced effect upon the subsequent staining of beta-cell granules by basic dyes. The dyes found to be most suitable for such staining after oxidation included basic fuchsin, paraldehyde-fuchsin, methyl violet 2-B, and crystal violet. Malachite green, methyl green, and spirit blue were less satisfactory. The optimal interval of staining with these dyes was 5 to 20 seconds, whereas in the case of paraldehyde-fuchsin without prior oxidation, the staining interval, though dependent upon the fixation employed, had to be considerably longer to give equivalent results. The basic fuchsin solution was effective only if used at a pH below 3; if the pH was 6.9, a differential staining of the islet-cell granules did not occur. Methyl violet 2-B and crystal violet worked effectively without adjusting the pH of their solutions. Both resulted in an intense metachromatic staining of the beta granules. This staining could be transformed into an orthochromatic coloration upon rapid dehydration of the sections in acetone. A slow dehydration of sections in acetone or ethanol resulted in complete extraction of the dye from the beta granules. Lowering the pH of the basic fuchsin, methyl violet 2-B, or crystal violet solutions did not affect the intensity of beta granule coloration, though the general background staining was suppressed.

Oxidation of sections in 1 per cent aqueous periodic acid for 2 or more hours at 25° C. or in 0.5 per cent aqueous acidified potassium permanganate for 5 to 10 minutes at 25° C., or bromination in bromine: carbon tetrachloride for 30 minutes at 25° C. produced results similar to those when peracetic acid was used for 2 to 3 minutes. Prolonging the oxidation or bromination interval, in general, accentuated the background staining.

Paraldehyde-fuchsin which had aged 2 months at 25° C. failed to stain the beta granules directly, though the elastic fibers were colored intensely. However, if the sections were first oxidized with peracetic acid, the beta granules were stained just about as quickly and intensely with old paraldehyde-fuchsin as with the relatively fresh dye.

No matter by which method the beta granules were stained, the granules were decolorized within 3 minutes with acid-alcohol (pH, 1.3), whereas the dye resisted extraction in a borax solution (pH, 9.2) for 30 minutes. The reverse occurred upon decolorizing the alpha-cell granules which had been stained by acid dyes, such as acid fuchsin. After decolorization, the granules were restainable in undiminished intensity, indicating that they did not dissolve in the solvent of the dye.

Staining Properties of Purified Insulin Crystals

Material. The insulin crystals used had been specially purified (The Lilly Research Laboratories, lot no. 466368).

Solubility of the Insulin Crystals. Approximately 20 mg. of the insulin crystals were found to be soluble at 25° C. in 20 ml. of each of the following solvents: N/10 NaOH in distilled water, N/10 HCl in distilled water or in 70 per cent ethanol, 1 per cent aqueous periodic acid, and peracetic acid. The dried insulin was found, under the same conditions, to be largely insoluble in distilled water (pH, 5.5), acetone, 95 per cent ethanol, and absolute ether.

Preparation of Insulin "Films." The "films" were fixed separately in the following fluids for 4 to 6 hours at 25° C.: (1) Bouin's picro-formalin acetic acid solution (pH, 1.4); (2) formol sublimate buffered with sodium acetate (pH, 5.8); and (3) 10 per cent formalin buffered with calcium acetate (pH, 6.9). The insulin "films" did not dissolve appreciably in these fixatives.

Staining of Fixed Insulin "Films." The fixed "films," after rinsing in distilled water, were stained separately in the following dye solutions for 45 seconds and then rinsed quickly in water: (1) 0.5 per cent basic fuchsin in 60 per cent ethanol (at pH 6.9, 2.5, and 1.3); (2) 0.5 per cent crystal violet in 60 per cent ethanol (at pH 5.5, 2.3, and 1.3); (3) 0.5 per cent acid fuchsin in 60 per cent ethanol (at pH 3.0, 2.2, and 1.3); (4) Gomori's paraldehyde-fuchsin, aged 48 hours (pH, 1.3).

The *results* are summarized as follows: Each dye solution, except paraldehyde-fuchsin, stained the insulin "films" orthochromatically and intensely regardless of the fixation employed or of the pH of the dye solution. The dyes obscured, without alteration of their natural color, the yellow coloration of the insulin-picrate (Bouin's fixed) "films." Paraldehyde-fuchsin failed to color the fixed "films" in 3 minutes, though at 5 minutes a dull pinkish tinge existed, and after 5 minutes the "films" dissolved in the dye solvent.

Oxidation with peracetic acid for 1 or more minutes led to dissolution of the fixed insulin "films."

Discussion

From the experimental data it appears that by peracetic acid or potassium permanganate oxidation and by bromination there is produced, in pancreatic beta-cell granules, an acid substance capable of *rapidly* taking up various basic dyes even from pH 1.3 solution. At this pH level, the staining of alpha-cell granules and acinar-cell cytoplasm is almost nil. Those basic dyes of strong affinity for the oxidized beta granules include basic fuchsin, paraldehyde-fuchsin, crystal violet, and methyl violet 2-B. The paraldehyde-fuchsin dye is unique in that it is capable of staining the beta granules selectively without the influence of prior oxidation, though the latter greatly accelerates the subsequent uptake of dye.

There is evidence, though indirect, that the above staining reactions depend upon the protein constituent of the beta-cell granules. So far as can be determined from various histochemical methods applied to frozen and paraffin sections, such granules do not possess lipid or carbohydrate components.[7]

The findings from the present investigation support the theory that the mechanism of staining pancreatic beta-cell granules is based upon those physicochemical factors affecting the interaction of basic dyes with proteins. Of importance in this regard is the reversible nature of the staining reaction when the solution environment of the tissue sections is changed Thus, beta-cell granules stained by a given basic dye can be decolorized readily with a solution free of dye if the pH of such solution is adjusted downward—a pH region which favors dissociation of a basic dye-protein combination. As observed in the present study, the extent and rate of destaining depends upon the particular dye and its affinity for the beta granules and the conditions of washing. These features appear to follow the general principles of staining tissue proteins as reviewed by Singer.[8]

It seems improbable that the staining reactions employed in the present investigation depend upon the formation of azomethines (Schiff's bases) between tissue aldehydes and basic dyes possessing open amino groups because of the following: (1) The beta granules fail to react with Schiff reagent either directly or after oxidation, indicating the absence of reactive aldehyde groups; (2) acid fuchsin, which possesses open amino groups, fails to stain the beta granules, whereas crystal violet, in which the amino groups are methylated, is capable of staining the beta granules after oxidation; (3) the formation of colored Schiff's bases in tissue sections is a slow process, whereas the staining of beta granules after oxidation is rapid; and (4) the use of

aldehyde blocking reagents (phenylhydrazine-HCl or aniline-HCl) fails to prevent the staining of the beta granules. A previous report[9] dealt with the physicochemical properties of basic fuchsin and of paraldehyde-fuchsin and with the conditions under which these dyes are capable of forming azomethines in tissue sections.

The problem now arises as to the nature of the protein substance characterizing the pancreatic beta-cell granules. Is it insulin or insulin precursor? Hartroft and Wrenshall[10] reported their finding of a high degree of correlation between bioassays of extractable insulin and numbers of beta-cell granules stained by paraldehyde-fuchsin. The material consisted of pancreases obtained at necropsy from diabetic and non-diabetic subjects. A loss in extractable insulin content, such as occurs in cases of diabetes mellitus, was found to parallel a loss in granulation of beta cells. An abnormal metabolic state in animals similar to diabetes mellitus in man may be produced experimentally in a variety of ways.[11] In such cases of experimental diabetes there also is an associated loss in beta-cell granulation.[11]

Thus, as is so often stated in recent literature, there is evidence that the beta-cell granules represent stored insulin or, at least, a precursor of insulin. If this is true, paraldehyde-fuchsin would be expected to react with a protein similar or identical to insulin. This hypothesis is controverted by the findings in the present investigation. Of importance are those observations dealing with the staining properties of purified insulin crystals in the form of insulin "films" subjected to the same fixations as applied to the pancreatic tissue. It was found that not only did paraldehyde-fuchsin fail to stain the fixed insulin "films" within 3 minutes, but also that after 5 minutes the insulin dissolved in the dye solvent. Furthermore, the fixed insulin "films" were stained rapidly by various basic dyes and by acid fuchsin regardless of the fixation employed or the pH of the dye solution, though at low pH levels the "films" eventually dissolved in the dye solvent. Such staining properties do not correspond with those of pancreatic beta-cell granules, though the dye solutions employed were identical.

Also of importance are those findings related to the solubility of fixed insulin "films" as compared to the solubility of pancreatic beta-cell granules fixed in an identical manner. As mentioned previously, the fixed insulin "films" dissolved in the dye solvent within a specified interval depending upon the pH of the solution, there being rapid dissolution in alcoholic solutions of low pH. The use of corresponding dye solutions did not result in dissolution of the fixed beta-cell granules even at prolonged intervals (24 to 48 hours). Furthermore, performic and peracetic acids are known to increase the solubility of

insulin by cleaving the cystine-disulfide bonds.[12] This results in the formation of two distinct polypeptide chains, each with characteristic solubility properties. In the present investigation purified insulin, whether subjected to fixation or not, dissolved rapidly in peracetic acid. In contrast, fixed pancreatic beta-cell granules did not appear altered in number or structure after tissue sections had been oxidized with peracetic acid for 24 hours, followed by washing in acid or alkaline solutions and then staining with paraldehyde-fuchsin or crystal violet. Thus, the solubility properties of purified insulin did not correspond with those of pancreatic beta-cell granules under the conditions employed.

It is evident, then, that pancreatic beta-cell granules do not represent stored insulin, assuming that the latter is in a form similar to extractable insulin. Yet, there remains to be explained the high degree of correlation between extractable insulin and numbers of beta-cell granules stained by paraldehyde-fuchsin in a particular pancreas. Since the nature of the protein characterizing the beta-cell granules is not known, it is impossible to determine the true significance of this granulation. At present, the degree of beta-cell granulation may be regarded as reflecting the state of biochemical activity for synthesizing insulin.

Summary

After specific oxidation or bromination, beta-cell granules in pancreatic islets were stained quickly and intensely by certain basic dyes, including paraldehyde-fuchsin. Factors that influenced such staining reactions are the type of fixation employed, the nature of the oxidant and interval of oxidation or bromination, the pH of the dye solution, the staining interval, and the use or omission of varied dehydrating agents after staining. Those basic dyes of strong affinity for the oxidized beta-cell granules included basic fuchsin, paraldehyde-fuchsin, crystal violet, and methyl violet 2-B. Paraldehyde-fuchsin was unique in that it stained the granules without the influence of prior oxidation, though the staining interval had to be considerably longer than if sections first were oxidized.

It appears that by oxidation with peracetic acid or potassium permanganate and by bromination with bromine:carbon tetrachloride there was produced, in beta-cell granules, an acid substance of protein nature capable of taking up rapidly and selectively certain basic dyes from pH 1.3 solution. At this pH level the staining of alpha-cell granules and acinar-cell cytoplasm was almost nil. The beta granules were decolorized readily and completely by acid-alcohol at pH 1.3, a pH region which favors dissociation of basic dye-protein combinations.

From the studies dealing with the staining and solubility properties of purified insulin crystals as compared to those properties of pancreatic beta-cell granules, it is concluded that the granules do not represent stored insulin.

Addendum

George Gomori, at the University of Chicago, reviewed this article shortly after it was accepted for publication. He made the comment that removal of paraldehyde-fuchsin from beta-cell granules by acid-alcohol does not occur unless the dye is freshly prepared. Since, in the present investigations, the paraldehyde-fuchsin dye was aged for only 48 hours, it was decided to try acid-alcohol destaining procedures on beta granules colored by paraldehyde-fuchsin aged for 8 days. In such instances, little or no dye was removed from the granules, thus confirming Gomori's findings. This indicates that the staining of beta-cell granules by paraldehyde-fuchsin does not depend upon ionic forces but is based upon a genuine chemical reaction between the dye and the protein in the granules.

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THE HISTOPATHOLOGY OF SWIMMERS' ITCH

I. The Skin Lesions of Schistosomatium douthitti and Gigantobilharzia huronensis in the Unsensitized Mouse*

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In 1928, Cort[1] demonstrated that non-human schistosome cercariae are capable of producing dermatitis in man. Since then, many species of avian and mammalian schistosomes have been shown to produce similar lesions, to which Cort gave the name, swimmers' itch. The gross lesions, as they appear in man and various species of experimental animals, have been well described, but the microscopic features of the host response have been the subject of only a few reports.

Vogel[2] (1930), working with *Cercaria pseudocellata,* described the response in human tissue taken for biopsy 24 hours after exposure. He noted moderate edema in the epidermis and a minimal cellular response consisting of neutrophils and lymphocytes. Dead cercariae were present in the epidermis.

Brackett[3] (1940), also reporting from human biopsy specimens, described the picture 29 hours after exposure to *C. stagnicolae,* and 50 hours after exposure to *C. elvae.* He noted marked edema and a neutrophilic response in the 29-hour biopsy material and a marked cellular exudate consisting principally of eosinophils and lymphocytes in that obtained at 50 hours.

Macfarlane[4] (1949), utilizing *C. longicauda,* described the host reaction from a series of human biopsies. In unsensitized individuals he found a mild response to the cercariae, consisting of edema, parakeratosis, and a lymphocytic exudate in the dermis. He was able to trace the fate of the cercariae from the time they first attacked the epidermis until they were killed and sloughed in an epithelial plaque with the stratum corneum about 2 weeks after exposure.

The response of the sensitized individuals in Macfarlane's series[4] showed edema and a massive lymphocytic exudate in both the dermis and epidermis. This reaction occurred in a much shorter period than did the milder response of the unsensitized persons.

In none of these three studies could the authors find any evidence that the cercariae had been able to penetrate the basal cell layer of the epidermis.

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Olivier and Weinstein[5] (1953) described the tissue response of rabbits to both *Trichobilharzia ocellata* and *T. stagnicolae*. The reaction of the rabbit to the two cercariae was similar, although more intense in the case of *T. ocellata*. The response of unsensitized rabbits was so minimal as not to be evident 48 hours after exposure. Living cercariae were observed in both the dermis and the epidermis. The response of the sensitized rabbits to these cercariae was intense and it consisted principally of leukocytes. The authors concluded that the cercariae may be destroyed by an undesignated humoral factor, by the cellular response, or by both.

Olivier[6] (1953) noted pulmonary hemorrhages in rabbits, mice, and several other laboratory animals that had been exposed to *T. ocellata*, demonstrating that avian schistosomes could proceed in mammals to a further stage of their life cycle than had been hitherto reported. Earlier, Penner[7] (1941) had shown that *Schistosomatium douthitti*, a parasite of muskrats and mice, could produce pulmonary hemorrhages in monkeys.

The gross lesions produced by schistosomes in abnormal hosts are apparently indistinguishable from one another, but the microscopic descriptions of these same lesions are too few to permit such a generalization. Furthermore, the exact method by which the host overcomes the cercariae is known only when the parasites are intraepidermal in location.

The present paper describes the response of the unsensitized white mouse to two species of schistosomes: *S. douthitti*, which is known to reach maturity in the mouse, and *Gigantobilharzia huronensis*, which has been described from several species of birds.[8]

Materials and Methods

Cercariae of *S. douthitti* and *G. huronensis* were used to infect white mice. Naturally infected *Lymnaea stagnalis*, collected from the muskrat farm of Mr. Charles Sagers of Fox Lake, Wisconsin, were the source of *S. douthitti;* and naturally infected *Physa gyrina*, collected from the Huron River near Ann Arbor, Michigan, were the source of *G. huronensis*. These snails were maintained in laboratory aquaria, to which calcium carbonate and lettuce were added periodically.

S. douthitti cercariae were collected by placing one or two *L. stagnalis* into a small glass vial, and the vial was then put in a darkened room. The cercariae, which are negatively phototropic, would begin to emerge from the snails, and within a short time they could be observed hanging from the surface film of water. Using a small hair loop, the

cercariae were picked off the surface film, were counted under a dissecting microscope, and were transferred to a 5 by 10 mm. glass vial. A mouse was anesthetized with phenobarbital and was taped to a small board. Its left ear was then dipped into the water containing the cercariae. The exposure of each mouse was to 100 ± 10 cercariae and was limited to 30 minutes.

The cercariae of *G. huronensis* are positively phototropic, and normally emerge from the snail in the early morning. These cercariae were collected by placing three or four *P. gyrina* into a small glass vial, and this vial was then placed in a darkened room for 24 to 30 hours prior to an exposure. The vials were then placed in a lighted room, at which time the cercariae would begin to emerge. These cercariae were surface hangers also, and were prepared for exposure in the manner described for *S. douthitti.*

At various intervals from the time of exposure, the mice were killed with phenobarbital and necropsied. The left ear and the internal organs were preserved in formalin. These tissues were prepared for microscopic examination by standard techniques and were sectioned at 5 μ. The ears were serially sectioned. All tissues were stained with hematoxylin and eosin and were mounted in Canada balsam.

Results

The Skin Lesions of S. douthitti

The gross pathologic features, as they appear in man and various species of experimental animals, have been well described by many investigators.[9] In the white mouse the cercariae of *S. douthitti* produced a maculopapular eruption. The macules were 0.3 mm. in diameter and appeared 5 to 10 minutes after an exposure. The papules appeared 30 to 60 minutes after exposure and disappeared completely during the third day following an exposure. At the height of the dermatitis the papules were 4 mm. in diameter and were surrounded by an erythematous ring approximately 5 mm. in diameter. If more than two or three cercariae had penetrated in close approximation, the epithelium would slough off, leaving an ulcer.

The microscopic picture of the host response varied with the fate of the cercariae. Many cercariae were able to penetrate only to the prickle-cell layer of the epithelium, where they produced intercellular and intracellular edema (Fig. 2). Occasionally there was also a minimal neutrophilic reaction in the dermis beneath, but usually there was none. The presence of the parasite seemed to induce hyperkeratosis of the epithelium beneath it (Fig. 2), resulting in the extrusion of the

cercaria with the exfoliating stratum corneum. Dead cercariae were seen in an epithelial plaque, forming part of the stratum corneum, within 90 minutes after exposure. This process was not observed after the third day following exposure.

Cercariae were observed to have penetrated into the dermis either directly through the basal layer of the epidermis (Fig. 3), or through the canal of the hair shaft into the sebaceous gland (Fig. 4) and thence into the dermis. In either case there was minimal neutrophilic response in the dermis in the immediate vicinity of the cercaria. When an ulcer had been produced by the entrance of several parasites within a small area of skin, there was a heavy neutrophilic response in the dermis, and often localized areas of hemorrhage (Fig. 5).

As soon as the cercariae had penetrated the dermis and subcutaneous tissues, tissue histiocytes began to accumulate in the vicinity of the parasites and to form a capsule around them (Figs. 6 to 8). Histiocytes were first observed about 12 hours after exposure. They reached maximum numbers about 4 days following exposure, after which cercariae were no longer seen in sections of the skin. The neutrophilic response remained moderate for as long as 3 weeks after exposure, whereas the histiocytic response subsided shortly after the cercariae disappeared from the skin and subcutaneous tissues.

A small number of living cercariae were observed in venules which were surrounded by minimal collections of both neutrophils and histiocytes (Figs. 9 and 10). Hemorrhage was observed throughout the site of the cercaria in these instances, as a result of the parasite's entry into the venule. The cecae of these cercariae were filled with golden-yellow or brown granules, the product of the parasite's metabolism.

The Skin Lesions of G. huronensis

In the majority of cases there were no gross lesions evident following exposure to *G. huronensis*. Infrequently, red macules about 0.3 mm. in diameter were observed. These appeared within 5 or 10 minutes and disappeared within 1 hour.

As with *S. douthitti*, these cercariae were observed to penetrate the dermis either directly through the basal cell layer of the epidermis or through the pilar apparatus into the sebaceous gland.

Microscopic sections showed that the cercariae were able to penetrate through the epidermis very rapidly. Living cercariae were observed in the subcutaneous tissues as soon as 15 minutes after exposure (Fig. 11). At this time they were beginning to be surrounded by tissue histiocytes, and there was a moderate histiocytic response throughout

the section. Within a very short time the cercariae were encapsulated by several layers of histiocytes in a manner similar to the process observed with *S. douthitti.* This local reaction persisted for 1 or 2 days, until the cercariae in the dermis and subcutaneous tissues were clearly dying or dead. It then disappeared quickly.

There was a minimal neutrophilic reaction noted 1 hour after exposure. This increased in intensity, reaching a maximum degree 1 day following exposure (Fig. 12). It gradually subsided, but it was still present 16 days after exposure.

A few living cercariae were observed in epidermal burrows without any sign of host reaction; and only one dead cercaria was seen in an epithelial plaque. No dead cercariae were observed within epithelial burrows. It was concluded, therefore, that practically all the cercariae that had attacked the skin had been able to penetrate the epidermis and to invade the subcutaneous tissues. This is in agreement with the minimal gross lesions.

Discussion

On the basis of the knowledge then available to him, Cort[9] (1950) stated that the dermatitis (in man) "produced by one species of non-human schistosome cercariae does not differ from that produced by any other." This is true in regard to the gross appearance of the dermatitis. Macfarlane[4] and Olivier[10] were able to demonstrate that the difference in the degree of the host response, and hence of the gross appearance of the lesions, was due solely to the previous degree of sensitization to schistosome cercariae which the host had experienced.

The investigations of Vogel,[2] Brackett,[3] and Macfarlane,[4] as well as the findings described in the present paper, all indicate that the cercariae which remain intra-epidermal cause a localized area of edema in the epithelial cells. This edema results in the development of a papule, which disappears as soon as the dead cercaria in the epithelial plaque is sloughed off with the stratum corneum. If the cercariae are able to penetrate through the epidermis, as in the case of *G. huronensis,* no papular dermatitis will be present. Upon the first exposure to schistosome cercariae, humans usually do not develop a dermatitis. It can be presumed that in these instances the cercariae are able to invade the subcutaneous tissues. Pulmonary migration of the non-human schistosomes in an unsensitized person, as suggested earlier by Olivier and Weinstein,[5] must be considered as a very distinct possibility.

As soon as the cercariae enter the dermis and subcutaneous tissues, there is an immediate local response by tissue histiocytes. The histiocytes serve to wall off the parasite, much as inert foreign bodies are

enveloped. The systemic response of the neutrophils is a more slowly developing process, and it becomes maximal only after a sufficient time has elapsed for the histiocytes completely to encapsulate the cercariae. The isolation of the cercariae in the subcutaneous tissues from their source of food contributes to the death of the parasites. The neutrophils then phagocytize the dead cercariae.

Living cercariae which have succeeded in entering venules were observed to induce a minimal reaction of histiocytes and neutrophils around the vessel. This is not an indication that the cercariae were dead, but rather that they had achieved this stage of their life cycle without succumbing to the host response.

Summary

The response of the unsensitized white mouse to cercariae of *Schistosomatium douthitti* and of *Gigantobilharzia huronensis* is very similar. The development of papules is caused by epithelial edema due to the cercariae which remain within the epidermis. Disappearance of the papules coincides with the extrusion of these dead cercariae in the stratum corneum.

The earliest demonstrable tissue reaction against cercariae in the dermis and subcutaneous tissues is a local accumulation of histiocytes. These histiocytes serve to encapsulate the parasites. Somewhat later there is a generalized neutrophilic response, which becomes maximal when the cercariae have become completely encapsulated. Phagocytosis of the parasites then ensues. Evidence of the inflammatory response was observed 3 weeks following exposure.

Addendum

After this paper had been accepted for publication, a similar study with *S. douthitti* was reported by Kagan and Meranze.[11]

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[*Illustrations follow*]

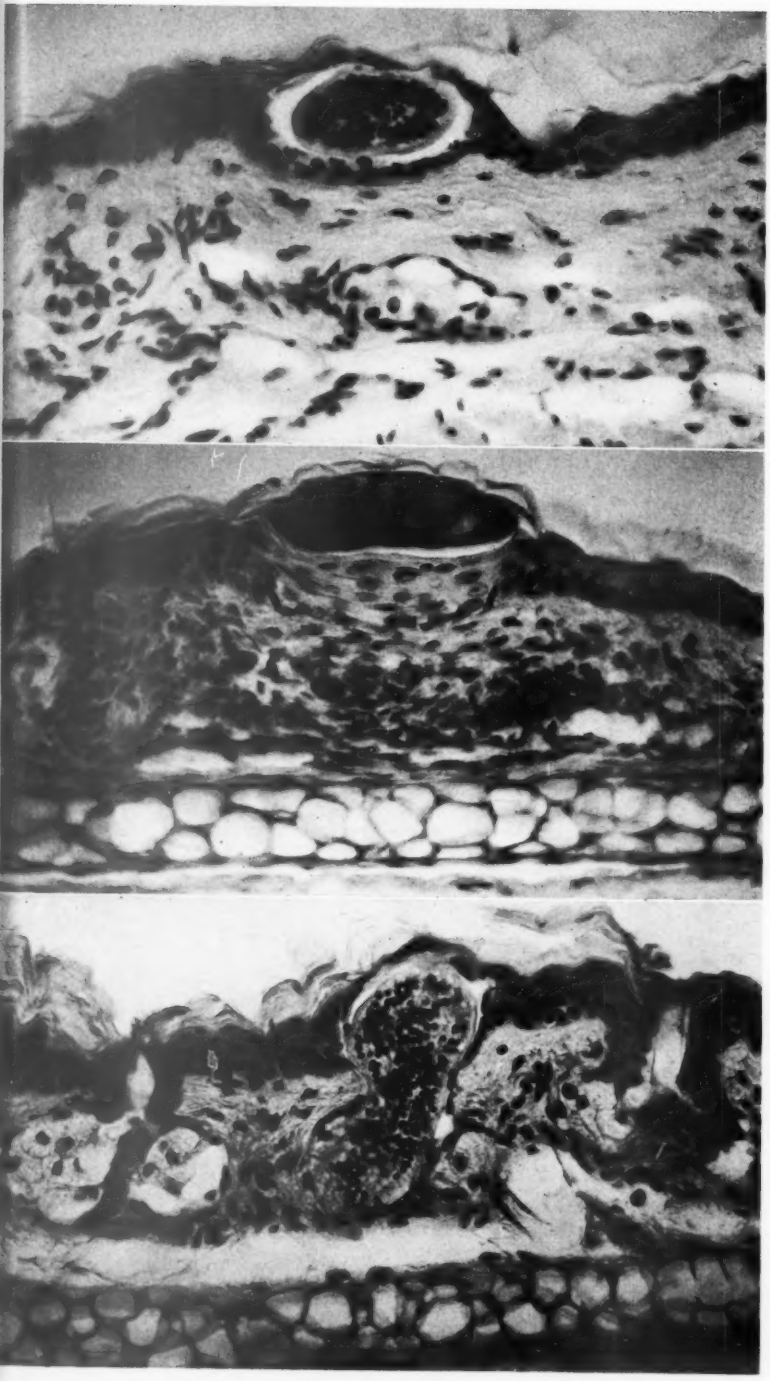
LEGENDS FOR FIGURES

All photographs were taken from sections of skin which were fixed in formalin and stained with hematoxylin and eosin. The magnification of Figures 1 to 4 and 6 to 12 is 275 ×, and that of Figure 5 is 140 ×. Figures 1 to 10 are after exposure to *Schistosomatium douthitti* and Figures 11 and 12 are after exposure to *Gigantobilharzia huronensis.*

FIG. 1. Living cercaria in epidermal burrow (2 hours after exposure). No evidence of host reaction.

FIG. 2. Dead cercaria in epidermal plaque (90 minutes after exposure) with moderate neutrophilic and very minimal histiocytic response in dermis. The epidermis is hyperkeratotic beneath the parasite.

FIG. 3. Living cercaria (2 hours after exposure) penetrating into the dermis directly through the epidermis. No host reaction is present.



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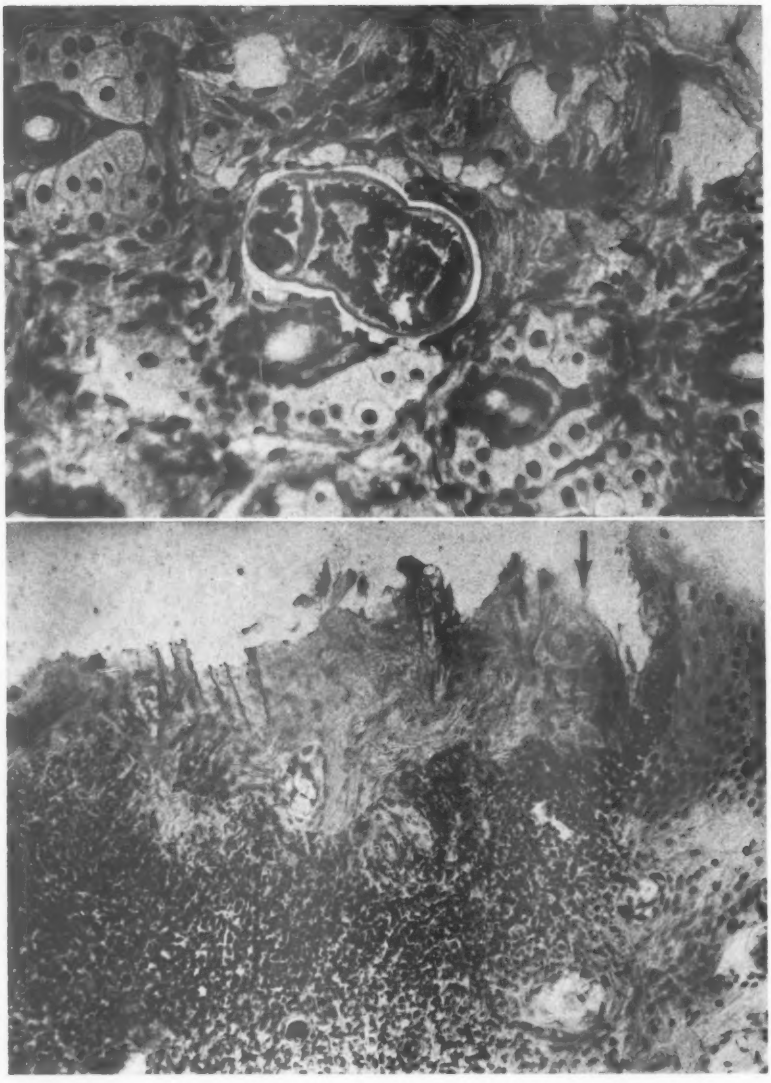
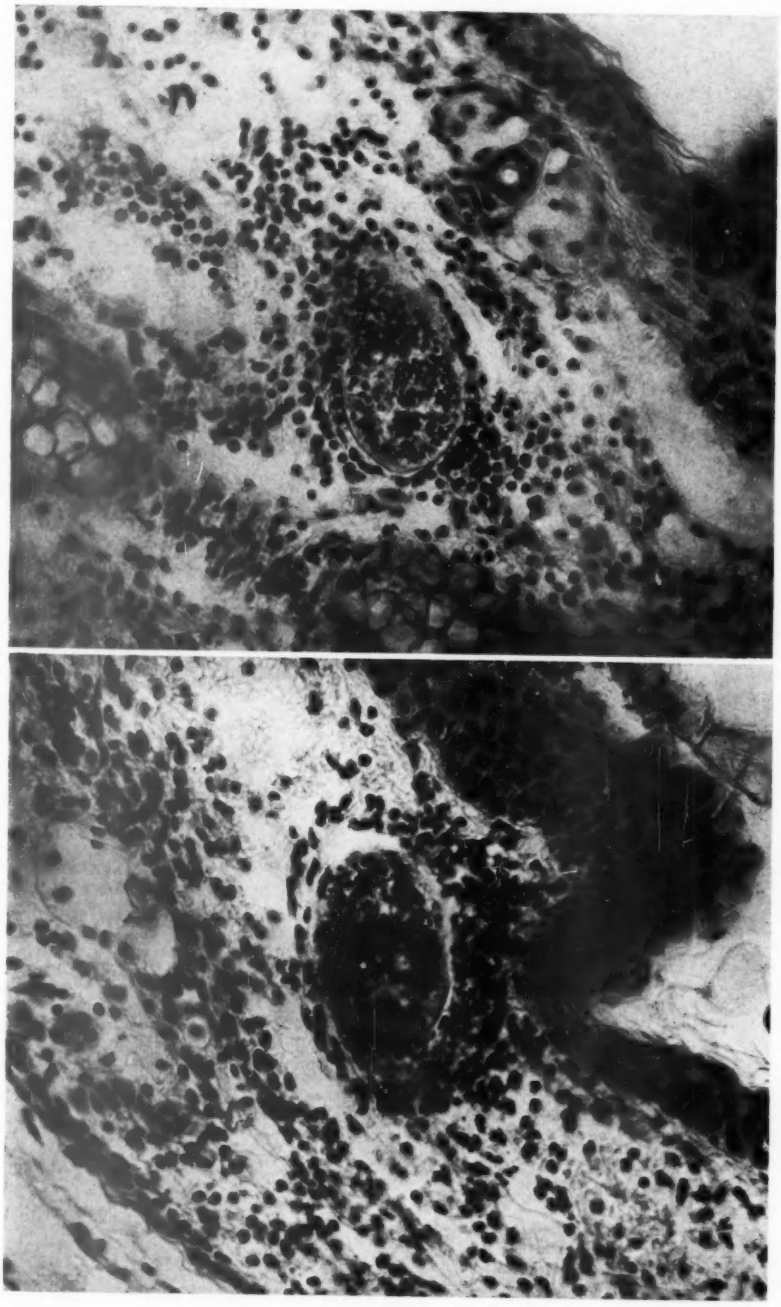


Fig. 4. Living cercaria (30 minutes after exposure) in sebaceous gland, without evidence of host reaction.

Fig. 5. Ulcer (20 hours after exposure) with heavy neutrophilic and minimal histiocytic reaction in dermis. There is a dead cercaria (arrow) in the base of the ulcer.

Fig. 6. Living cercaria (20 hours after exposure) with histiocytes beginning to surround it. Moderate neutrophilic and histiocytic reaction in the section.

Fig. 7. Dead cercaria (30 hours after exposure) in a later stage of encapsulation. Moderate neutrophilic and histiocytic reaction in the section.



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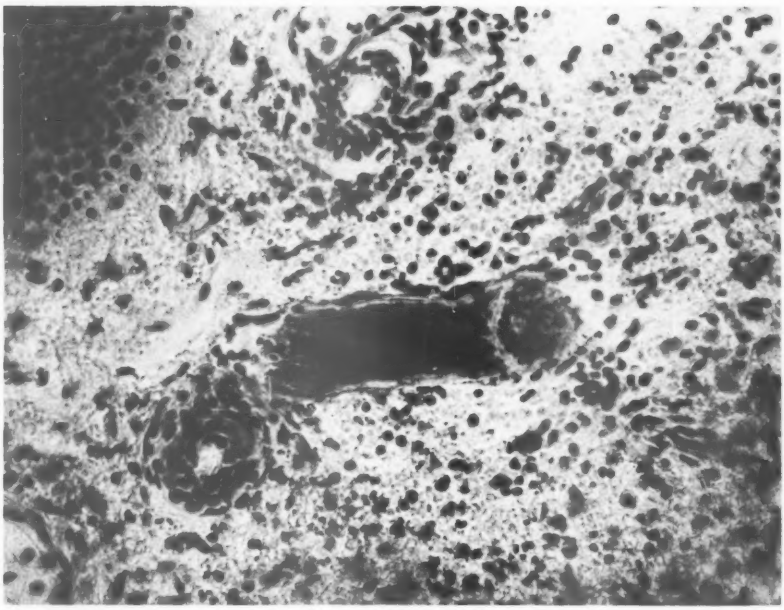
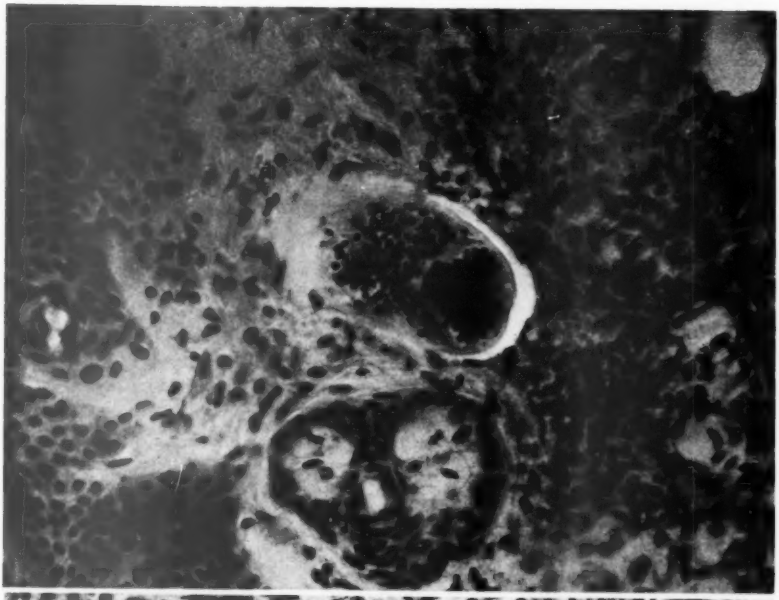


Fig. 8. Dead cercaria (30 hours after exposure) with a moderate neutrophilic and histiocytic reaction. Hemorrhage is present, and the cecae of the parasite are filled with pigment granules.

Fig. 9. Living cercaria (4 days after exposure) within a venule. Mild neutrophilic and histiocytic reaction in the section.

Fig. 10. Living cercaria (4 days after exposure) within a venule. Moderate neutrophilic and minimal histiocytic reaction around the venule.



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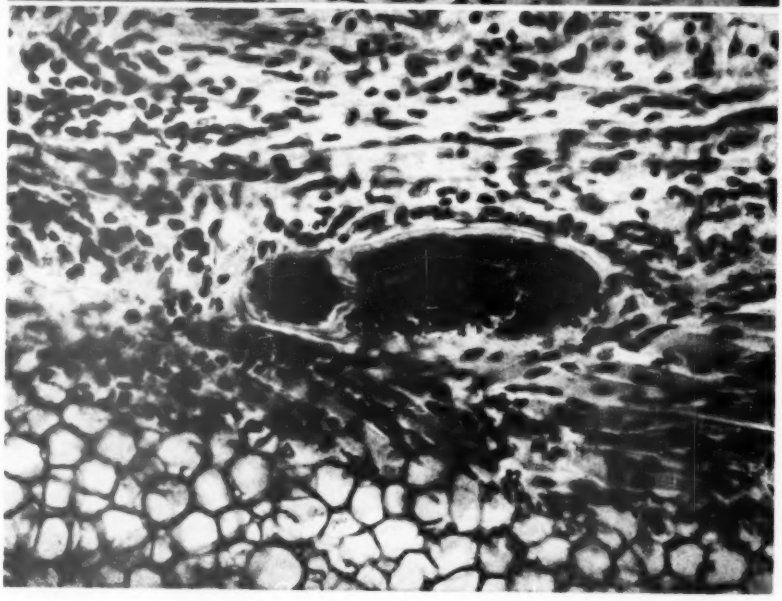

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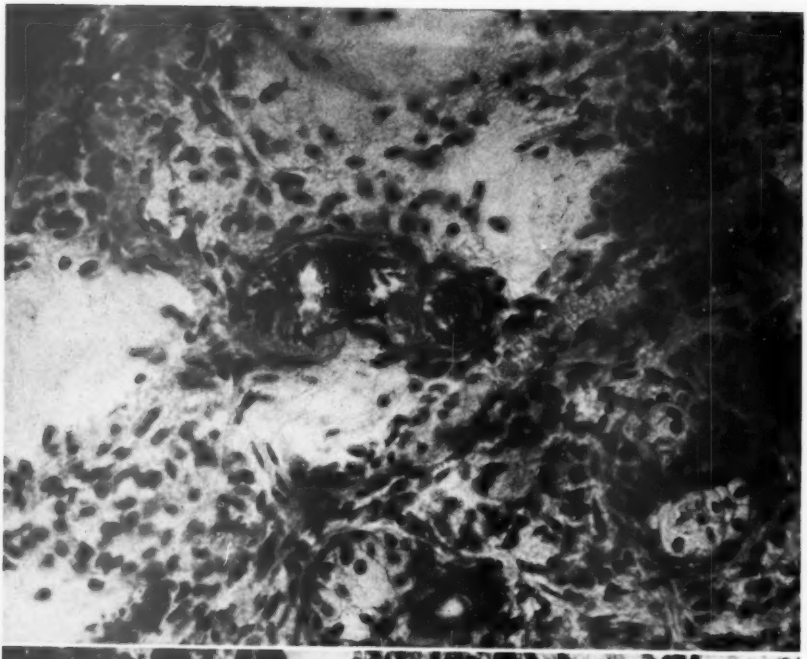
FIG. 11. Living cercaria (15 minutes after exposure) with a minimal histiocytic reaction around the parasite. There is no evidence of a neutrophilic response at this time.

FIG. 12. In the lower center of the field there is a dead cercaria in the subcutaneous tissues (1 day after exposure), with a heavy neutrophilic and moderate histiocytic reaction. The parasite is phagocytized.



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